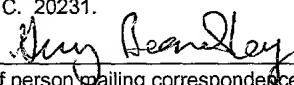


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09/709201

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UTILITY PATENT APPLICATION TRANSMITTAL UNDER 37 CFR §1.53(b)	
Attorney Docket Number	50150/007002
Applicant	William M. Mitchell et al.
Title	DIAGNOSIS AND MANAGEMENT OF INFECTION CAUSED BY CHLAMYDIA
PRIORITY INFORMATION:	
This application is a continuation of and claims priority from United States patent application 09/025,521, filed February 18, 1998.	
APPLICATION ELEMENTS:	
Cover sheet	1 page
Specification	81 pages
Claims	13 pages
Abstract	1 page
Drawing	4 sheets
Combined Declaration and POA, which is: <input checked="" type="checkbox"/> unsigned; <input type="checkbox"/> Newly signed for this application; <input type="checkbox"/> A copy from prior application [**SERIAL NUMBER**] and the entire disclosure of the prior application is considered as being part of the disclosure of this new application and is hereby incorporated by reference therein.	2 pages
Statement Deleting Inventors	[**] pages
Sequence Statement	2 pages
Sequence Listing on Paper	43 pages
Sequence Listing on Diskette	[**] disk
Small Entity Statement, which is: <input type="checkbox"/> Unsigned; <input type="checkbox"/> Newly signed for this application; <input checked="" type="checkbox"/> Two copies from prior application 09/025,521 and such small entity status is still proper and desired.	2 pages
Preliminary Amendment	5 pages

09709201 11/08/00

IDS	2 pages
Form PTO 1449	1 page
Cited References	12 references
Recordation Form Cover Sheet and Assignment	5 pages
A copy of a Petition for Extension of Time from prior application 09/025,521.	1 page
Return Receipt Postcard	1

Basic Filing Fee: \$355.00	\$355.00
Excess Claims Fee: 9 -20 x\$9	\$0
Excess Independent Claims Fee: 5 -3 x \$40	\$80.00
Multiple Dependent Claims Fee: \$270/\$135	\$
Total Fees:	\$435.00

- CORRESPONDENCE ADDRESS:**

Telephone: 617-428-0200
Facsimile: 617-428-7045

~~Signature~~

James DuCamp
Reg. No. 43,580

Date _____

November 8, 2000

VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) & 1.27(d))-NONPROFIT ORGANIZATION

DOCKET NUMBER: VDB96-02pA2

Applicant or Patentee: William M. Mitchell and Charles W. Stratton

Serial or Patent No.: 09/025,521

Filed or Issued: February 18, 1998

Title: DIAGNOSIS AND MANAGEMENT OF INFECTION CAUSED BY CHLAMYDIA

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF NONPROFIT ORGANIZATION Vanderbilt University

ADDRESS OF NONPROFIT ORGANIZATION 405 Kirkland Hall

Nashville, Tennessee 37203

TYPE OF NONPROFIT ORGANIZATION:

☒ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION

☐ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 U.S.C. 501(a) and 501(c)(3))

☐ NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA
(NAME OF STATE _____)

(CITATION OF STATUTE _____)

☐ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 U.S.C. 501(a) and 501(c)(3)) IF
LOCATED IN THE UNITED STATES OF AMERICA

☐ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED
STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA

(NAME OF STATE _____)

(CITATION OF STATUTE _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for
purposes of paying reduced fees to the United States Patent and Trademark Office regarding the invention described in:

☐ the specification filed herewith with title as listed above.

☒ the application identified above.

☐ the patent identified above.

I hereby declare the rights under contract or law have been conveyed to and remain with the nonprofit organization regarding the above
identified invention. If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights in the
invention must file separate verified statements averring to their status as small entities and that no rights to the invention are held by any person,
other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any
concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization having any rights in the invention is listed below:

☐ no such person, concern, or organization exists.

☒ each such person, concern or organization is listed below.

Merlin Technologies, Inc.

39 Karen Road

Newton, Massachusetts 02168

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity
status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small
entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are
believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are
punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may
jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Larry R. Steranka, Ph.D.

TITLE IN ORGANIZATION OF PERSON SIGNING Director, Office of Technology Transfer

ADDRESS OF PERSON SIGNING Vanderbilt University, 110 21st Avenue South, Nashville, Tennessee 37203

SIGNATURE Larry R. Steranka

DATE June 4, 1998

VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS

DOCKET NUMBER: VDB96-02pA2

(37 CFR 1.9(d) & 1.27(c))-SMALL BUSINESS CONCERN

Applicant or Patentee: William M. Mitchell and Charles W. Stratton

Serial or Patent No.: 09/025,521

Filed or Issued: February 18, 1998

Title: DIAGNOSIS AND MANAGEMENT OF INFECTION CAUSED BY CHLAMYDIA

I hereby declare that I am

☐ the owner of the small business concern identified below:

☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF SMALL BUSINESS CONCERN Merlin Technologies, Inc.

ADDRESS OF SMALL BUSINESS CONCERN 39 Karen Road

Newton, Massachusetts 02168

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.12 and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention described in:

☐ the specification filed herewith with title as listed above.

☒ the application identified above.

☐ the patent identified above.

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights in the invention must file separate verified statements averring to their status as small entities, and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization having any rights in the invention is listed below:

☐ no such person, concern, or organization exists.

☒ each such person, concern or organization is listed below.

Vanderbilt University

405 Kirkland Hall

Nashville, Tennessee 37240

Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Richard W. Berenson

TITLE OF PERSON IF OTHER THAN OWNER President

ADDRESS OF PERSON SIGNING 39 Karen Road, Newton, Massachusetts 02168

SIGNATURE 

DATE June 28, 1998

AOC1/WP1/DOC/VDB9602pA2.VRM

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Guy Beardsley

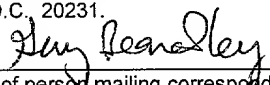
Printed name of person mailing correspondence

Guy Beardsley

Signature of person mailing correspondence

APPLICATION
FOR
UNITED STATES LETTERS PATENT

APPLICANT : WILLIAM M. MITCHELL AND CHARLES W. STRATTON
TITLE : DIAGNOSIS AND MANAGEMENT OF INFECTION
CAUSED BY CHLAMYDIA

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<u>Guy Beardsley</u> Printed name of person mailing correspondence	 Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: William M. Mitchell et al. Art Unit:
Serial No.: Examiner:
Filed: November 8, 2000
Title: DIAGNOSIS AND MANAGEMENT OF INFECTION CAUSED BY
CHLAMYDIA

Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, kindly amend the application as follows.

In the specification:

At page 1, replace the cross-reference to related applications with the following:

--This application is a continuation application of U.S. serial no. 09/025,521 (now pending), hereby incorporated by reference.--.

At page 34, line 16, replace "_____" with --09/025,596--.

09/025,596

At page 34, line 18, delete “(Attorney Docket No. VDB98-01).”

In the claims:

Kindly cancel claims 1-47 and 49-67, and amend claim 48 as follows.

48. (Amended) A method of detecting *Chlamydia* [chlamydial elementary bodies] in a sample, said method comprising performing on the sample an antigen capture assay using an antibody that specifically binds to a peptide having a sequence consisting essentially of SEQ ID NO: 93; SEQ ID NO: 96; SEQ ID NO: 97; SEQ ID NO: 100; or SEQ ID NO: 101 [directed against MOMP or the peptides described in Figures 3, 4, and 5].

Kindly add new claims 68-75 as follows.

--68. A method for detecting *Chlamydia* in a sample, said method comprising the steps of:

a) contacting said sample with an antibody that specifically binds to a peptide having a sequence consisting essentially of SEQ ID NO: 93; SEQ ID NO: 96; SEQ ID NO: 97; SEQ ID NO: 100; or SEQ ID NO: 101; and

b) detecting the binding of said antibody to a sample component of said sample, wherein binding of said antibody indicates the presence of *Chlamydia* in said sample.

69. The method of claim 68, wherein said antibody specifically binds to a peptide having a sequence consisting essentially of SEQ ID NO: 93 or SEQ ID NO: 97, and the binding of said antibody to said sample component indicates that said *Chlamydia* is *Chlamydia pneumoniae*.

70. The method of claim 68, wherein said antibody specifically binds to a peptide having a sequence consisting essentially of SEQ ID NO: 96 or SEQ ID NO: 100, and the binding of said antibody to said sample component indicates that said *Chlamydia* is *Chlamydia psittaci*.

71. A method for detecting the presence of *Chlamydia* in a sample, said method comprising the steps of:

- a) immobilizing said sample onto a substrate;
- b) contacting said sample with an antibody that specifically binds to a peptide having a sequence consisting essentially of SEQ ID NO: 93; SEQ ID NO: 96; SEQ ID NO: 97; SEQ ID NO: 100; or SEQ ID NO: 101, wherein said antibody becomes immobilized if said sample contains *Chlamydia*; and
- c) detecting the presence of immobilized antibody, wherein the presence of immobilized antibody indicates the presence of *Chlamydia* in said sample.

72. The method of claim 71, wherein said antibody specifically binds to a peptide having a sequence consisting essentially of SEQ ID NO: 93 or SEQ ID NO: 97, and the presence of immobilized antibody indicates that said *Chlamydia* is *Chlamydia pneumoniae*.

73. The method of claim 71, wherein said antibody specifically binds to a peptide having a sequence consisting essentially of SEQ ID NO: 96 or SEQ ID NO: 100, and the presence of immobilized antibody indicates that said *Chlamydia* is *Chlamydia psittaci*.

74. A substantially pure polypeptide having a sequence consisting essentially of SEQ ID NO: 93; SEQ ID NO: 96; SEQ ID NO: 97; SEQ ID NO: 100; or SEQ ID NO: 101.

75. An antibody that specifically binds to a peptide having a sequence consisting essentially of SEQ ID NO: 93; SEQ ID NO: 96; SEQ ID NO: 97; SEQ ID NO: 100; or SEQ ID NO: 101.--

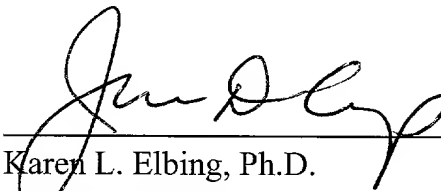
Support for new claims 68-75 is found at pages 67-73 of the specification. No new matter has been added by this amendment.

Conclusion

Enclosed is a copy of the petition to extend the period for replying to the Examiner's action in parent application serial no. 09/025,521 for three months, to and including November 8, 2000. If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: November 8, 2000



Karen L. Elbing, Ph.D.
Reg. No. 35,238

Clark & Elbing LLP
176 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045

James DeCamp
Reg. No. 43,580

Inventors: William M. Mitchell and Charles W. Stratton
Attorney's Docket No.: VDB96-02pA2

RELATED APPLICATIONS

BACKGROUND OF THE INVENTION

EBs are small (300-400 nm) infectious, spore-like forms which are metabolically inactive, non-replicating,

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and found most often in the acellular milieu. EBs are resistant to a variety of physical insults such as enzyme degradation, sonication and osmotic pressure. This physical stability is thought to be a result of extensive disulfide cross-linking of the cysteine-rich major outer membrane protein (MOMP) (Bavoil et al., *Infection and Immunity*, 44:479-485 (1984); Hackstadt et al., *Journal of Bacteriology*, 161:25-31 (1985); Hatch et al., *Journal of Bacteriology*, 165:379-385 (1986); Peeling et al., *Infection and Immunity*, 57:3338-3344 (1989); J.C.A. Bardwell, *Molecular Microbiology*, 14:199-205 (1994); and T.P. Hatch, *Journal of Bacteriology*, 178:1-5 (1993)). Under oxidizing conditions in the acellular milieu of the host, the outer membrane of EBs is relatively impermeable as well as resistant to inactivation. EBs are thus well suited to survive long enough outside of their hosts to be transmitted to a new host in the form of a droplet nuclei (Theunissen et al., *Applied Environmental Microbiology*, 59:2589-2593 (1993)) or a fomite (Fasley et al., *The Journal of Infectious Diseases*, 168:493-496 (1993)).

Infection by members of the genus *Chlamydiae* induces a significant inflammatory response at the cellular level. For example, genital lesions produced by *Chlamydia trachomatis* frequently elicit a vigorous influx of lymphocytes, macrophages, and plasma cells, suggesting the development of humoral and cellular immunity. Yet, clinically, the initial infection is frequently varied in symptomatology and may even be asymptomatic. Once fully established, the *Chlamydia* are difficult to eradicate, with frequent relapse following antibiotic therapy. Evidence also indicates that the *Chlamydia* may become dormant and are then shed in quantities too few to reliably detect by culture.

Chlamydia pneumoniae (hereinafter "*C. pneumoniae*") is the most recent addition to the genus *Chlamydiae* and is

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isolated from humans and currently is recognized as causing approximately 10 percent of community acquired cases of pneumonia (Grayston et al., *J. Inf. Dis.* 161:618-625 (1990)). This newly recognized pathogen commonly infects the upper and lower respiratory tract and is now recognized as ubiquitous in humans. *C. pneumoniae* is the most recent addition to the genus *Chlamydiae* and is well-accepted as a human pathogen that may be difficult to eradicate by standard antibiotic therapy (Hammerschlag et al., *Clin. Infect. Dis.* 14:178-182 (1992)). *C. pneumoniae* is known to persist as a silent or mildly symptomatic pathogen, resulting in a chronic, persistent infection (J. Schacter, In: Baun AL, ed. *Microbiology of Chlamydia*, Boca Raton, FL, CRC Press, 1988, pp. 153-165).

The current therapy for suspected/confirmed *C. pneumoniae* infection is with a short course (e.g., 2-3 weeks) of a single antibiotic. *C. pneumoniae* is susceptible *in vitro* to tetracycline, erythromycin, clarithromycin, and fluoroquinolones such as ofloxacin and sparfloxacin (Kuo et al., *Antimicrob Agents Chemother* 32:257-258 (1988); Welsh et al., *Antimicrob Agents Chemother* 36:291-294 (1992); Chirgwin et al., *Antimicrob Agents Chemother* 33:1634-1635 (1989); Hammerschlag et al., *Antimicrob Agents Chemother* 36:682-683 (1992); Hammerschlag et al., *Antimicrob Agents Chemother* 36:1573-1574 (1992); M.R. Hammerschlag, *Antimicrob Agents Chemother* 38:1873-1878 (1994); M.R. Hammerschlag, *Infect. Med.* pp. 64-71 (1994)). Despite this demonstration of *in vitro* susceptibility, *C. pneumoniae* infections may relapse following antibiotic therapy with these agents. *In vitro* studies on the persistence of *Chlamydiae* despite specific and appropriate antibiotic therapy have suggested that the presence of antibiotics promotes the formation of an intracellular, non-replicative state (Beatty et al., *Microbiol. Rev.* 58:686-699 (1994)), typically referred to as the latent or

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cryptic phase. This change can be thought of as a stringent response and is seen also with nutrient starvation and exposure to γ -interferon. Removal of the stressful influence allows the organism to resume replication. Thus, in this way, the organism can escape current antibiotic therapy used in clinical practice.

In view of the chronic and persistent nature of chlamydial infections, there is a need for reliable, accurate methods for diagnosis of pathogenic infection as well as therapeutic approaches to manage the infection. Due to the highly infective nature of *Chlamydia* EBs and their ability to reinfect cells, there is also a need for antichlamydial therapy which totally eradicates this pathogen, thereby preventing the long term sequelae of such chronic infections.

SUMMARY OF THE INVENTION

The present invention provides a unique approach for the diagnosis and management of infection by *Chlamydia* species, particularly *C. pneumoniae*. The invention is based upon the discovery that a combination of agents directed toward each of the various stages of the chlamydial life cycle can successfully manage infection and ultimately prevent reinfection/reactivation of the pathogen. Accordingly, one embodiment of the invention pertains to methods of treating infection by a *Chlamydia* species, comprising administering to an individual in need thereof a combination of antichlamydial agents, comprising at least two agents, each of which is effective against a different phase of the chlamydial life cycle. For example, the method can be carried out using agents chosen from among the following groups: a) at least one agent effective against the elementary body phase of the chlamydial life cycle; b) at least one agent effective against the replicating phase of the chlamydial life cycle; and c) at

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The invention also pertains to a novel therapy comprising a specific agent effective against the elementary body phase of the chlamydial life cycle which, if used for a sufficient period of time, allows active

-6-

infection to be completed without the creation of infectious EBs.

In order to facilitate patient compliance during a course of therapy, the invention provides a means for packaging therapeutic agents, described herein, for the management of *Chlamydia* infection. For example, a pack can comprise at least two different agents, each of which is effective against a different phase of the chlamydial life cycle. These agents can be selected from the group consisting of: a) at least one agent effective against the elementary body phase of the chlamydial life cycle; b) at least one agent effective against the replicating phase of the chlamydial life cycle; and c) at least one agent effective against the cryptic phase of the chlamydial life cycle. Optional adjunct compounds, as mentioned previously, can likewise be present in the pack. A preferred pack will comprise a plurality of agents that are directed to at least two, but preferably to all, of the stages of the chlamydial life cycle. The pack can provide a unit dosage of the agents or can comprise a plurality of unit dosages, and may be labeled with information, such as the mode and order of administration (e.g., separate, simultaneous or sequential) of each component contained therein.

The invention also encompasses a method for evaluating the infection status of an individual and/or the progress of therapy in an individual undergoing therapy for infection caused by *Chlamydia*. The method comprises quantifying antibody titer or other measure to the pathogen and comparing the measure to antibody measure quantified at a time earlier in the therapy, whereby the difference between the measures is indicative of the progress of the therapy. The invention also pertains to a method for monitoring the course of therapy for treating infection by *Chlamydia*, comprising determining presence or absence of

-7-

Chlamydia in an infected individual at time intervals during course of therapy. In a particular embodiment, this is determined by PCR assay or antigen capture assay for pathogen DNA.

5 Detection of the presence of *Chlamydia* in a sample of biological material taken from an individual thought to be infected therewith is important in determining the course of therapy and the agents to be used. This can be achieved by detecting the presence of DNA encoding MOMP of *Chlamydia*
10 or other chlamydial genes in the individual. In one aspect of the invention, diseases associated with *Chlamydia* infection, such as inflammatory diseases, autoimmune diseases and diseases in which the individual is immunocompromised, can be treated by managing (e.g.,
15 significantly reducing infection or eradicating) the *Chlamydia* infection using the novel approach described herein. Both clinical and serological improvements/resolutions in patient status have been demonstrated.

20 The invention also pertains to a susceptibility test for identifying agent(s) capable of significantly reducing/eliminating chlamydial infection. The method comprises preparing tissue culture from cell lines; inoculating these cells with *Chlamydia* in the absence of cycloheximide; allowing the *Chlamydia* to infect these cells
25 for several days; adding agent(s) to be tested, which agent(s) is/are replaced as needed for the duration of incubation; isolating chlamydial nucleic acid from the cells; and assessing the presence or absence of chlamydial DNA using a suitable nucleotide amplification assay, such
30 as PCR. Preferably the presence or absence of signal for amplified DNA encoding MOMP of *Chlamydia* or other chlamydial protein is determined. Absence of a signal indicates a reduction in the degree of infection below that which is detectable by nucleic acid amplification
35 techniques and strongly suggests eradication of the

-8-

microorganism. The susceptibility tests described herein are particularly useful as a drug screening tool for assessing the activity of single agents or combinations of agents against *Chlamydia* infection.

5 The unique and novel aspect of the susceptibility test described herewithin is that it measures the presence or absence of chlamydial DNA and thus can detect cryptic forms and/or elementary bodies both of which are infectious, yet are not replicating.

10 In one embodiment, a suitable nucleotide assay for identifying agents effective against the cryptic form of chlamydia comprises, in the presence of agent(s) to be tested, subjecting cultured cells to protease/reducing agent (e.g., dithiotreitol (DTT)) and protease digestion or
15 guanidine isothiocyanate (also known as guanidine thiocyanate) for a prescribed period of time; extracting DNA from the treated solution; exposing DNA to appropriate polymerase, dNTPs and primers for DNA amplification of MOMP or other protein of the *Chlamydia* species; and determining
20 the presence or absence of amplified DNA by visualizing the ethidium bromide treated DNA product by gel electrophoresis, for example. In particular embodiments, the *Chlamydia* species is *C. pneumoniae* and the appropriate primers are CHLMOMPDB2 and CHLMOMPCB2.

25 The invention further relates to a method of identifying cells containing the cryptic form of a *Chlamydia* species by a nucleic acid amplification technique (e.g., PCR) comprising subjecting cultured cells to protease digestion; stopping protease activity; exposing
30 cells to appropriate heat-stable DNA polymerase, dNTPs and labeled primers (e.g., 3'-biotin labeled, 5'-biotin labeled) for amplification of DNA encoding MOMP of the *Chlamydia* species; washing the cells; exposing the cells to a reporter molecule (e.g., strepavidin-conjugated signal
35 enzyme); exposing the cells to an appropriate substrate for

-9-

the reporter molecule (e.g., conjugated enzyme); and visualizing the amplified DNA encoding MOMP by visualizing the product of the reaction.

A method of identifying cells containing the cryptic form of *Chlamydia* comprises treating cultured cells, thought to be infected with *Chlamydia*, with a disulfide reducing agent; subjecting cultured cells to protease digestion; exposing cells to appropriate polymerase, dNTPs and primers for DNA amplification of nucleic acid encoding a chlamydial protein; exposing the cells to a reporter molecule enzyme; exposing the cells to an appropriate substrate for the reporter enzyme; and determining the presence of the cryptic form of *Chlamydia* by visualizing the amplified DNA encoding a chlamydial protein. Preferably the amplification technique is PCR and the primers are CHLMOMPDB2 and CHLMOMPCB2 of *Chlamydia pneumoniae*.

A similar method can be used as an assay for identifying an agent which is effective against the cryptic form of *Chlamydia*. Accordingly, the method comprises treating cultured cells grown in the absence of cycloheximide, thought to be infected with *Chlamydia*, with a disulfide reducing agent; allowing the chlamydia to replicate; adding a test agent; subjecting cultured cells to protease digestion; exposing cells to appropriate polymerase, dNTPs and primers for DNA amplification of a chlamydial protein; exposing the cells to a reporter molecule enzyme; exposing the cells to an appropriate substrate for the reporter enzyme; and determining the presence of cryptic form of *Chlamydia* by visualizing the amplified DNA encoding a chlamydial protein, such as MOMP.

Also described is a method of detecting chlamydial elementary bodies in a sample comprising contacting the sample with a disulfide reducing agent before using a DNA

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have been cleared of *Chlamydia* infection by the methods of this invention, or have never been infected, such as their *Chlamydia*-free offspring or progeny. Cells or animals can be maintained as *Chlamydia*-free by maintaining them on

5 antibiotics and/or treating their nutrients and environment to ensure that they are *Chlamydia*-free. Particularly, a source of nutrients to be administered to *Chlamydia*-free cells or animals can be treated to inactivate or remove any chlamydial elementary bodies therefrom. This can be

10 accomplished by exposing the nutrients to gamma irradiation for a period of time and level of exposure adequate to inactivate the elementary bodies. In addition to, or alternatively, a source of nutrients can be passed through a filtration system to physically remove the chlamydial

15 elementary bodies therefrom. Optionally, the source of nutrients can be first treated with a disulfide reducing agent, such as dithiothreitol, before the filtration step is performed. The filter should be of adequate size such that objects larger than 0.5 microns are prevented from

20 passing through.

The invention further pertains to a diagnostic kit or pack comprising an assembly of materials selected from the group consisting of antibiotics, reagents, *Chlamydia*-free cell lines, and combinations thereof, or other materials

25 that would be necessary to perform any of the methods described herein.

The invention further relates to a method of detecting viable *Chlamydia* in a biological material suspected of being contaminated therewith, comprising culturing

30 *Chlamydia*-free cells or animals in the presence of biological material and then determining the presence or absence of viable *Chlamydia* in the culture.



Figure 2 shows the expressed thioredoxin fusion protein containing a polyhistidine affinity chromatography site, an enterokinase cleavage site, and the full length MOMP protein with an alanine insertion after aa1. Amino to carboxyl reads left to right. Total amino acid content in the expressed protein is 530 residues.

Figure 4 illustrates the peptide amino acid sequences employed for the construction of peptide based ELISAs with species specificity for VD1.

DETAILED DESCRIPTION OF THE INVENTION

Diagnostic and therapeutic methods for the management of *Chlamydia* infections are described in detail below. For the purposes of this invention, "management of *Chlamydia*

Diagnostic and therapeutic methods for the management of *Chlamydia* infections are described in detail below. For the purposes of this invention, "management of *Chlamydia*

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infection" is defined as a substantial reduction in the presence of all phases/forms of *Chlamydia* in the infected host by treating the host in such a way as to minimize the sequellae of the infection. *Chlamydia* infections can thus be managed by a unique approach referred to herein as "combination therapy" which is defined for the purpose of this application as the administration of multiple agents which together are directed at least two but preferably each of the multiple phases of the chlamydial life cycle, each agent taken separately, simultaneously or sequentially over the course of therapy. When used alone, these agents are unable to eliminate chlamydial infection. The diagnostic methods and combination therapies described below are generally applicable for infection caused by any *Chlamydia* species, such as *C. pneumoniae*, *C. trachomatis*, *C. psittaci* and *C. pecorum*. Infections in which the causative agent is *C. pneumoniae* are emphasized.

Antichlamydial agents, which have been identified as effective against *Chlamydia* by the susceptibility testing methods described herein, can be used singly or in combination to manage *Chlamydia* infection. For example, compounds identified as anti-cryptic phase drugs, anti-EB phase drugs, anti-DNA-dependent RNA polymerase drugs and nicotinic acid co-gener drugs can be used alone or in combination to eliminate, reduce or prevent one or more of the distinct phases of the chlamydial life cycle. These compounds have not heretofore been shown to have antichlamydial activity.

DIAGNOSIS OF CHLAMYDIA INFECTION

The invention pertains to methods for diagnosing the presence of *Chlamydia* in a biological material, as well as to the use of these methods to evaluate the serological status of an individual undergoing antichlamydial combination therapy. For purposes of this application,

"biological material" includes, but is not limited to, bodily secretions, bodily fluids and tissue specimens. Examples of bodily secretions include cervical secretions, trachial-bronchial secretions and pharyngeal secretions.

5 Suitable bodily fluids include blood, sweat, tears, cerebral spinal system fluid, serum, urine, synovial fluid and saliva. Animals, cells and tissue specimens such as from a variety of biopsies are embraced by this term.

10 In one embodiment, peptide-based assays are disclosed for the detection of one or more immunoglobulins, such as IgG, IgM, IgA and IgE, against antigenic determinants within the full length recombinant MOMP of various *Chlamydia* species. Detection of IgG and/or IgM against antigenic determinants within the the full length
15 recombinant MOMP of *C. pneumoniae* is preferred. IgA determinations are useful in the analysis of humoral responses to *Chlamydia* in secretions from mucosal surfaces (e.g., lung, GI tract, genitourinary tract, etc.). Similarly, IgG determinations are useful in the analysis of
20 allergic manifestations of disease. Table 1 below provides the GenBank Accession numbers of various MOMPs for *Chlamydia* species.

00909601-10800

Table 1

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Species	Strain	ID	GenBank Accession No.
C. trachomatis	A	CTL/A	M33636
C. trachomatis	A	CTL/A	M58938 M33535
C. trachomatis	A	CTL/A	J03813
C. trachomatis	B	CTL/B	M33636
C. trachomatis	C	CTL/L	M17343 M19128
C. trachomatis	D	CTL/D	A27838
C. trachomatis	E	CTL/E	X52557
C. trachomatis	F	CTL/F	X52080 M30501
C. trachomatis	H	CTL/H	X16007
C. trachomatis	L1	CTL/L1	M36533
C. trachomatis	L2	CTL/L2	M14738 M19126
C. trachomatis	L3	CTL/L3	X55700
C. trachomatis	Mouse Pneumo	CTL/MP	X60678
C. pecorum	Ovine Polyarthrititis	CPC/OP	Z18756
C. psittaci	Strain 6BC	CPS/6B	X56980
C. psittaci	Feline	CPS/F	X61096
C. trachomatis	Da	CTL/DA	X62921 S45921
C. pneumoniae	TWAR	CPN/HU1	M64064 M34922 M64063
C. pneumoniae (? C. pecorum)	Horse	CPN/EQ2	L04982
C. pneumoniae	TWAR	CPN/MS	not assigned
C. psittaci	Horse	CPS/EQ1	L04982

For example, a biological material, such as a sample of tissue and/or fluid, can be obtained from an individual and a suitable assay can be used to assess the presence or amount of chlamydial nucleic acids or proteins encoded

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thereby. Suitable assays include immunological methods such as enzyme-linked immunosorbent assays (ELISA), including luminescence assays (e.g., fluorescence and chemiluminescence), radioimmunoassay, and immunohistology.

- 5 Generally, a sample and antibody are combined under conditions suitable for the formation of an antibody-protein complex and the formation of antibody-protein complex is assessed (directly or indirectly). In all of the diagnostic methods described herein, the antibodies can
10 be directly labeled with an enzyme, fluorophore, radioisotope or luminescer. Alternatively, antibodies can be covalently linked with a specific scavenger such as biotin. Subsequent detection is by binding avidin or strepavidin labeled with an indicator enzyme, fluorophore,
15 radioisotope, or luminescer. In this regard, the step of detection would be by enzyme reaction, fluorescence, radioactivity or luminescence emission, respectively.

- The antibody can be a polyclonal or monoclonal antibody, such as anti-human monoclonal IgG or anti-human
20 monoclonal IgM. Examples of useful antibodies include mouse anti-human monoclonal IgG that is not cross reactive to other immunoglobulins (Pharmagen; Clone G18-145, Catalog No. 34162D); mouse anti-human monoclonal IgM with no cross reactivity to other immunoglobulins (Pharmagen; Clone G20-
25 127, Catalog No. 34152D). Peptide-based immunoassays can be developed which are *Chlamydia* specific or provide species specificity, but not necessarily strain specificity within a species, using monoclonal or polyclonal antibodies that are not cross-reactive to antigenic determinants on
30 MOMP of a chlamydial species not of interest.

- Recombinant-based immunological assays have been developed to quantitate the presence of immunoglobulins against the *Chlamydia* species. Full length recombinant *Chlamydia* MOMP can be synthesized using an appropriate
5. expression system, such as in *E. coli* or Baculovirus. The

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expressed protein thus serves as the antigen for suitable immunological methods, as discussed above. Protein-based immunological techniques can be designed that are species- and strain-specific for various *Chlamydia*.

- 5 Diagnosis of chlamydial infection can now be made with an improved IgM/IgG *C. pneumoniae* method of quantitation using ELISA techniques, Western blot confirmation of ELISA specificity and the detection of the MOMP gene of *C. pneumoniae* in serum using specific amplification primers
- 10 that allow isolation of the entire gene for analysis of expected strain-specific differences.

- Any known techniques for nucleic acid (e.g., DNA and RNA) amplification can be used with the assays described herein. Preferred amplification techniques are the
- 15 polymerase chain reaction (PCR) methodologies which comprise solution PCR and *in situ* PCR, to detect the presence or absence of unique genes of *Chlamydia*. Species-specific assays for detecting *Chlamydia* can be designed based upon the primers selected. Examples of suitable PCR
- 20 amplification primers are illustrated below in Table 2. Examples of preferred primers are illustrated in Table 3.

"SECRET" 10250260

Table 2
Initial and Terminal Nucleotide Sequences of Chlamydial MOMP Genes
in which entire sequence is known

GenBank Accession No.	ID	Initial Fifty Nucleotides	SEQ ID NO.
M64064/M34922/M64063	CPNHU1	ATGAAAAAACTCTTAAAGTCGGCGTTATTATCCGCGCATTTGCTGGTTC	1
None	CPNHU2 ^a	ATGAAAAAACTCTTAAAGTCGGCGTTATTATCCGCGCATTTGCTGGTTC	2
L04982	CPNEQ1	ATGAAAAAACTCTTGAAGTCGGCATTTATTGTTTGCGCTACGGGTTCCGC	3
L04982	CPNEQ2	ATGAAAAAACTCTTAAAGTCGGCGTTATTATCCGCGCATTTGCTGGTTC	4
X56980	CPS/6B	ATGAAAAAACTCTTGAATTCGGCATTTATTGTTTGCGCTACGGGTTCCGC	5
M36703	CPS/AB1	ATGAAAAAACTCTTGAATTCGGCATTTATTGTTTGCGCTACGGGTTCCGC	6
L39020	CPS/AB2	ATGAAAAAACTCTTGAATTCGGCATTTATTGTTTGCGCTACGGGTTCCGC	7
L25436	CPS/AV/C	ATGAAAAAACTCTTGAATTCGGCATTTATTGTTTGCGCTACGGGTTCCGC	8
X61096	CPS/F	ATGAAAAAACTCTTGAATTCGGCATTTATTGTTTGCGCTACGGGTTCCGC	9
M33636/N58938/J03813	CTL/A	ATGAAAAAACTCTTGAATTCGGTATTAGTATTTGCGGCTTTGAGTTCTGC	10
M17343/M19128	CTL/C	ATGAAAAAACTCTTGAATTCGGTATTAGTATTTGCGGCTTTGAGTTCTGC	11
X62921/S45921	CTL/DA	ATGAAAAAACTCTTGAATTCGGTATTAGTATTTGCGGCTTTGAGTTCTGC	12
X52557	CTL/E	ATGAAAAAACTCTTGAATTCGGTATTAGTATTTGCGGCTTTGAGTTCTGC	13
X52080/M30501	CTL/F	ATGAAAAAACTCTTGAATTCGGTATTAGTATTTGCGGCTTTGAGTTCTGC	14
X16007	CTL/H	ATGAAAAAACTCTTGAATTCGGTATTAGTATTTGCGGCTTTGAGTTCTGC	15
M36533	CTL/L1	ATGAAAAAACTCTTGAATTCGGTATTAGTATTTGCGGCTTTGAGTTCTGC	16
M14738/M19126	CTL/L2	ATGAAAAAACTCTTGAATTCGGTATTAGTATTTGCGGCTTTGAGTTCTGC	17
X55700	CTL/L3	ATGAAAAAACTCTTGAATTCGGTATTAGTATTTGCGGCTTTGAGTTCTGC	18
X60678	CTL/MP	ATGAAAAAACTCTTGAATTCGGTATTAGTATTTGCGGCTTTGAGTTCTGC	19

Variable	Mean	SD	Min	Max
Age	38.5	12.5	18	65
Gender	0.5	0.5	0	1
Marital status	0.7	0.5	0	1
Education	12.5	2.5	9	16
Income	15.5	10.5	5	35
Occupation	1.5	1.5	1	5
Health status	1.5	1.5	1	5
Stress level	2.5	1.5	1	5
Life satisfaction	3.5	1.5	1	5
Depression	1.5	1.5	1	5
Anxiety	1.5	1.5	1	5
Resilience	3.5	1.5	1	5
Optimism	3.5	1.5	1	5
Self-efficacy	3.5	1.5	1	5
Perceived stress	2.5	1.5	1	5
Life events	1.5	1.5	1	5
Support system	3.5	1.5	1	5
Coping strategies	3.5	1.5	1	5
Quality of life	3.5	1.5	1	5
Healthcare utilization	1.5	1.5	1	5
Health insurance	0.5	0.5	0	1
Access to care	1.5	1.5	1	5
Health literacy	3.5	1.5	1	5
Health beliefs	3.5	1.5	1	5
Health behaviors	3.5	1.5	1	5
Health outcomes	3.5	1.5	1	5
Health equity	3.5	1.5	1	5
Health disparities	1.5	1.5	1	5
Health determinants	3.5	1.5	1	5
Health interventions	3.5	1.5	1	5
Health policies	3.5	1.5	1	5
Health systems	3.5	1.5	1	5
Health research	3.5	1.5	1	5
Health education	3.5	1.5	1	5
Health promotion	3.5	1.5	1	5
Health communication	3.5	1.5	1	5
Health behavior change	3.5	1.5	1	5
Health equity interventions	3.5	1.5	1	5
Health disparities research	3.5	1.5	1	5
Health determinants research	3.5	1.5	1	5
Health interventions research	3.5	1.5	1	5
Health policies research	3.5	1.5	1	5
Health systems research	3.5	1.5	1	5
Health research methods	3.5	1.5	1	5
Health education research	3.5	1.5	1	5
Health promotion research	3.5	1.5	1	5
Health communication research	3.5	1.5	1	5
Health behavior change research	3.5	1.5	1	5
Health equity interventions research	3.5	1.5	1	5
Health disparities research methods	3.5	1.5	1	5
Health determinants research methods	3.5	1.5	1	5
Health interventions research methods	3.5	1.5	1	5
Health policies research methods	3.5	1.5	1	5
Health systems research methods	3.5	1.5	1	5
Health research ethics	3.5	1.5	1	5
Health education ethics	3.5	1.5	1	5
Health promotion ethics	3.5	1.5	1	5
Health communication ethics	3.5	1.5	1	5
Health behavior change ethics	3.5	1.5	1	5
Health equity interventions ethics	3.5	1.5	1	5
Health disparities research ethics	3.5	1.5	1	5
Health determinants research ethics	3.5	1.5	1	5
Health interventions research ethics	3.5	1.5	1	5
Health policies research ethics	3.5	1.5	1	5
Health systems research ethics	3.5	1.5	1	5
Health research ethics training	3.5	1.5	1	5
Health education ethics training	3.5	1.5	1	5
Health promotion ethics training	3.5	1.5	1	5
Health communication ethics training	3.5	1.5	1	5
Health behavior change ethics training	3.5	1.5	1	5
Health equity interventions ethics training	3.5	1.5	1	5
Health disparities research ethics training	3.5	1.5	1	5
Health determinants research ethics training	3.5	1.5	1	5
Health interventions research ethics training	3.5	1.5	1	5
Health policies research ethics training	3.5	1.5	1	5
Health systems research ethics training	3.5	1.5	1	5
Health research ethics certification	3.5	1.5	1	5
Health education ethics certification	3.5	1.5	1	5
Health promotion ethics certification	3.5	1.5	1	5
Health communication ethics certification	3.5	1.5	1	5
Health behavior change ethics certification	3.5	1.5	1	5
Health equity interventions ethics certification	3.5	1.5	1	5
Health disparities research ethics certification	3.5	1.5	1	5
Health determinants research ethics certification	3.5	1.5		

a Sequence from a cerebral spinal fluid of a patient with multiple sclerosis isolated by the inventors.
Sequence is identical to TWAR C. pneumoniae with exception of a C/T mutation at NT 54 and a G/A mutation at NT 126.

b Terminator codon underlined

b
at NI 128.
Terminator condon underlined

Table 3
Primers for PCR Amplification of Entire MOMP Gene ^a

<i>Chlamydia</i>			Plus Strand Primer		SEQ ID NO.
Species	Strain	ID	Sequence	T _m ^b	
<i>C. pneumoniae</i>	TWAR	CHLMOMP DB2	ATGAAAAAAC TCTTAAAGTC GGCATTATTA TCCGCCGC	61.4°	105
<i>C. trachomatis</i>	L2	CTMOMP L2DB	ATGAAAAAAC TCTTGAATC GGTATTAGTG TTTGCCGCTT TGAG	61.2°	106
<i>C. psittaci</i>	Feline	PSOMP FPN-D	ATGAAAAAAC TCTTAAATC GGCATTATTA TTTGCCGCTG CGGG	62.1°	107
<i>C. psittaci</i>	6BC	PSOMP 6BC-b	ATGAAAAAAC TCTTGAATC GGCATTATTG TTTGCCGCTA CGGG	63.0°	108
<i>C. trachomatis</i>	Mouse	CTMU MOMP-D	ATGAAAAAAC TCTTGAATC GGTATTAGCA TTTGCCGCTT TGGGTTCTGC	63.5°	109

Table 3 (Continued)
Primers for PCR Amplification of Entire MOMP Gene ^a

Chlamydia			Minus Strand Primer		SEQ ID NO.
Species	Strain	ID	Sequence	T _m ^b	
<i>C. pneumoniae</i>	TWAR	CHLMOMP CB2	TTAGAATCTG AACTGACCAG ATACGTGAGC AGCTCTCTCG	64.4°	110
<i>C. trachomatis</i>	L2	CTMOMP L2CB	TTAGAAAGCG AANTGTGCAT TTACGTGAGC AGCTC	61.5°	111
<i>C. psittaci</i>	Feline	PSOMP FPN C	TTAGAATCTG AANTGAGCAT TAATGTGAGC AGCTCTTTCG TCG	62.2°	112
<i>C. psittaci</i>	6BC	PSOMP GBC C	TTAGAATCTG AANTGACCAT TCATGTGAGC AGCTCTTTCA TTGATTAAGC G	63.4°	113
<i>C. trachomatis</i>	Mouse	CTMU MOMP C	TTAGAAACGG AACTGAGCAT TTACGTGAGC TGCTCTTTCA TC	63.2°	114

^a All primers amplify under identical amplification conditions: 94°C for 1 min., 58°C for 2 min., 74°C for 3 min., for 35 cycles with 72°C for 10 min. extension of last cycle.

^b Melting temperature in degrees Celsius of a nucleic acid isomer based on the equation of Mermur and Doty (*J. Mol. Biol.* 5: 109-118, 1962) where $T_m = 81.5 + 16.6 \log_{10} (Na^+/K^+) + 41 (GC) - 600/L$ where (Na^+/K^+) in the molar cation concentration, GC in the mole fraction of GC and L is the sequence fragment length. (Na^+/K^+) used for computation was 0.05M.

Ligase chain reaction can also be carried out by the methods of this invention; primers/probes therefor can be constructed using ordinary skill. Amplification of the entire MOMP gene is useful for mutational analysis and the production of recombinant MOMP. Shorter primers can be used for specific amplification of most of the MOMP genome with a modification of amplification protocol. For example, a 22bp negative strand primer of the sequence 5'-CAGATACGTG AGCAGCTCTC TC-3' (CPNMOMPC; SEQ ID NO. 39) with a computed $T_m = 55^\circ$ plus a 25bp positive strand primer of the sequence 5'-CTCTTAAAGT CGGCGTTATT ATCCG-3' (CPNMOMPD; SEQ ID NO. 40) with a computed $T_m = 53.9^\circ$ can be used as a primer pair by adjusting the hybridization step in the amplification protocol (Table 2) from 58°C to 50°C .

Similarly, smaller regions of MOMP can be amplified by a large variety of primer pairs for diagnostic purposes although the utility of strain identification is reduced and amplification may be blocked if one or both primer pairs hybridize to a region that has been mutated.

Extensive experience with the full length MOMP PCR amplification indicates that mutational events within the CHLMOMPD32 and CHLMOMPCB2 hybridization sites are rare or non-existent.

The nucleic acid amplification techniques described above can be used to evaluate the course of antichlamydial therapy. The continued absence of detectable chlamydial DNA encoding MOMP as a function of antichlamydial therapy is indicative of clinical management of the chlamydial infection. Serological improvement can be based upon the current serological criteria for eradication of chronic *Chlamydia* reported below in Table 4.

Table 4

Serological Criteria for Eradication
of Chronic *Chlamydia pneumoniae* Infection

5

IgM	≤1:25
IgG	Stable titer 1:100
PCR	Negative

Preferred PCR techniques are discussed in detail below in the Example Section. In general, solution PCR is carried out on a biological material by first pre-incubating the material in an appropriate reducing agent that is capable of reducing the disulfide bonds which maintain the integrity of the MOMP and other surface proteins of the chlamydial elementary bodies, thereby compromising the outer protective shell of the EBs and allowing protease penetration. Suitable disulfide reducing agents include, but are not limited to, dithiothreitol, succimer, glutathione, DL-penicillamine, D-penicillamine disulfide, 2,2'-dimercaptodipic acid, 2,3-dimercapto-1-propone-sulfide acid. Appropriate concentrations of these reducing agents can be readily determined by the skilled artisan without undue experimentation using a 10 μ M concentration of dithiothreitol (the preferred reducing agent) as a guideline. Failure to include a reducing agent in the initial step may prevent DNA of EBs from being isolated in the subsequent step. Data presented in Example 1 shows the effects of various reducing agents on the susceptibility of EBs to proteinase K digestion. The *in vitro* data shows that dithiothreitol is most effective at opening EBs for protease digestion.

Once the outer shell of the EBs has been released, the pre-incubated material is subjected to protein digestion using a protease (e.g., proteinase K), or functionally

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equivalent enzyme. The DNA is extracted and subjected to a nucleic acid amplification technique, e.g., PCR. The entire gene or portion thereof containing unique antigenic determinant(s) encoding MOMP or other suitable gene can then be amplified using appropriate primers flanking the gene to be amplified. For example, the gene or portion thereof can be the gene encoding MOMP, OMP-B, GRO-ES, GRO-EL, DNAK, 16S RNA, 23S RNA, the gene encoding ribonuclease-P 76 kd attachment protein or a KDO-transferase gene. In an alternative method, guanidine thiocyanate, at preferably a concentration of 4M, or functionally equivalent reducing denaturant may be substituted for the disulfide reduction/protease steps.

The amplified DNA is then separated and identified by standard electrophoretic techniques. DNA bands are identified using ethidium bromide staining and UV light detection. PCR primers can be designed to selectively amplify DNA encoding MOMP of a particular *Chlamydia* species, such as the MOMP of *C. pneumoniae*, *C. pecorum*, *C. trachomatis*, *C. psittaci* (See Figure 1). Primers that are from about 15-mer to about 40-mer can be designed for this purpose.

For *in situ* PCR, the amplification primers are designed with a reporter molecule conjugated to the 5'-terminus. Suitable reporter molecules are well known and can be used herein. However, biotin-labeled primers are preferred. For the MOMP gene, the primers CHLMOMPDB2 and CHLMOMPCB2 have been engineered with a biotin at the 5'-terminus. For *in situ* PCR, using biotin labels incorporated at the 5'-terminus of the amplification primers, each DNA chain amplification results in each double strand DNA containing 2 molecules of biotin. Alternatively, other specific DNA sequences can be used, although the above-described sequence is the preferred embodiment since the large product produced (1.2 kb)

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prevents diffusion that may be encountered with smaller DNA amplifications. Similarly, other detection labels can be incorporated (i.e., fluorescein, for example) at the 5'-end or digoxigenin-dUTP (replacement for dTTP) can be incorporated within the amplified DNA. Alternatively to labeling the product, specific hybridization probes to constant regions of the amplified DNA can be used to identify an amplified product. This latter method has particular utility for the construction of automated laboratory equipment for solution-based PCR. For example, streptavidin-coated ELISA plates can be used to capture one or both strands of a biotin 5'-labeled DNA with detection by fluorescence of a fluorescein or other incorporated fluorophore detection probe.

15 CLEARING AND MAINTAINING CHLAMYDIA-FREE ORGANISMS

The present invention provides a unique approach for creating and maintaining animals and cell lines which are free of *Chlamydia* infection. Also described herein are methods for creating nutrients and culture media that are suitable for use with animals and cell lines that have been cleared of *Chlamydia* infection.

Attempts to culture isolates of *C. pneumoniae* from blood and cerebrospinal fluid (CSF) have resulted in the discovery that the continuous cell lines routinely used to cultivate *C. pneumoniae* are cryptically infected with *C. pneumoniae*. These include not only in house stocks of HeLa, HL, H-292, HuEVEC and McCoy cells, but also stocks obtained from the American Type Culture Collection (ATCC), The University of Washington Research Foundation for HL cells, as well as a commercial supplier (Bartells) of H-292 and McCoy cells for the clinical culture of *Chlamydia*. The presence of a cryptic form of *C. pneumoniae* in these cells has been repeatedly demonstrated by solution PCR amplifying the MOMP. *In situ* PCR in HeLa cells against the MOMP

demonstrates the MOMP genes to be present in 100% of cells. Nevertheless, fluoroscenated mAb to LPS in McCoy cells does not yield any indication of *Chlamydia* (i.e., reactive against all *Chlamydia*) while fluoroscenated mAb to C.

5 *pneumoniae* MOMP yields a generalized fluorescence throughout the cytoplasm that can be confused with non-specific autofluorescence. Infection with *Chlamydia trachomatis* (Bartells supply) yields the typical inclusion body staining with the LPS mAb (i.e., cross reactive with
10 all species of *Chlamydia*) with no change in cytoplasmic signal with anti-MOMP mAb against *C. pneumoniae*. These findings (solution PCR, *in situ* PCR, mAb reactivity) were interpreted as consistent with a cryptic (non-replicating) infection by *C. pneumoniae* of cells commonly used to
15 culture the organism. Further, virtually all rabbits and mice tested to date have PCR signals for the *C. pneumoniae* MOMP gene.

This creates a currently unrecognized problem of major significance for those clinical labs providing C.
20 *pneumoniae* culture services as well as investigators who now do not know whether their results in animals or in cell culture will be affected by cryptic chlamydial contamination. Clinical and research laboratories currently have no way to determine whether an organism is,
25 in fact, *Chlamydia*-free.

This invention pertains to a method for clearing cells and animals of *C. pneumoniae* and keeping them clear. Clearing them entails contacting the infected organism with agents used singly or in combination to eliminate or
30 interfere with more than one of the distinct phases of the life cycle of *Chlamydia* species. Keeping them clear entails either maintaining them on antibiotics and/or treating their nutrients and environment to ensure they are *Chlamydia*-free. In a preferred embodiment, maintenance
35 conditions comprise a combination of isoniazid (INH) (1

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µg/ml), metronidazole (1 µg/ml), and dithiothreitol (10 µM) in the culture medium. Media changes are accomplished every 3 days or twice per week. The cells can be removed from the protective solution between 1 and 7 days before they are to be used for culture or other purpose.

These techniques have now made it possible to create a variety of *Chlamydia*-free (CF) organisms, including continuous cell lines called HeLa-CF, HL-CF, H-292-CF, HuEVEC-CF, McCoy-CF, African green monkey and other cell lines that are capable of supporting chlamydial growth. Various CF strains of mice, rabbits and other animal models for research use can be produced.

Because *Chlamydia* is highly infectious, organisms which have been cleared of extracellular, replicating and cryptic infections must be protected from exposure to viable EBs if the organisms are to remain clear. The inventors have discovered that many of the nutrients and other materials used to maintain continuous cell lines are contaminated with viable *Chlamydia* EBs. For example, every lot of fetal calf serum has tested positive for the *Chlamydia* MOMP gene by PCR. Since extensive digestion is required for isolation of the DNA, we have concluded it is bound in EBs. *C. pneumoniae* can also be cultured directly from fetal calf serum. Thus, it is necessary to inactivate EBs in these materials, such as culture media and nutrients, used to maintain the *Chlamydia*-free status of the organism. Collectively these materials are referred to herein as "maintenance materials". In one embodiment, nutrients and culture media are subjected to gamma irradiation to inactivate *Chlamydia* therein. Preferably, the material should be irradiated for a period of time sufficient to expose the material to at least 10,000 rads of gamma radiation. It is important for the material to be contained in vessels that do not absorb high energy radiation. The preferred vessel is plastic. In another



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SUSCEPTABILITY TESTING FOR EVALUATING ACTIVE AGENTS AGAINST
VARIOUS FORMS OF CHLAMYDIA

30

The inventors have discovered that successful eradication of chronic/systemic chlamydial infections can be predicted by using the described unique methods for *in vitro* and *in vivo* susceptibility testing.

5 The invention is based upon the discovery that current susceptibility testing methods for *Chlamydiae* do not accurately predict the ability of antimicrobial agents to successfully and totally eradicate chronic chlamydial infections. This is because the current susceptibility
10 testing methods measure only replication of chlamydia and ignores the well-known "cryptic phase" (28-33) in which *Chlamydiae* are not actively replicating. Moreover, it has also been discovered that the so-called "cryptic phase" of *Chlamydiae* includes multiple and different phases. The
15 following are phases of the chlamydial life cycle in which the *Chlamydiae* are not replicating: an initial intranuclear phase in which elementary bodies (EBs) transition to reticulate bodies (RBs), an intracytoplasmic phase in which there is a transition of the RB phenotype to the EB
20 phenotype, an intracytoplasmic phase with a nonreplicating, but metabolizing RB, and intracellular/extracellular EB phases in which there is neither replication nor metabolism. In order to assess the cumulative and long term effect of antimicrobial therapy on these multiple life
25 phases, unique *in vitro* and *in vivo* susceptibility test methods have been developed and are described herein.

 The term "susceptibility" as used herein is intended to mean a physiological response of an organism to an environmental or chemical stimuli. The desired
30 physiological response to stimuli is one which adversely affects the pathogen's viability to replicate or reside within the host cell and, ideally, would result in the complete elimination (i.e., death) of that pathogen.

00309901-110000

A. *In Vitro* Methodology

One aspect of the invention pertains to methods for evaluating the susceptibility of the distinct phases and stages of the life cycle of *Chlamydia*, to a particular agent(s), particularly the cryptic phase, since prior techniques have failed, heretofore, to appreciate the need for drugs that can clear infected cells of cryptic *Chlamydia*. A preferred drug screening method which accomplished this objective utilizes tissue culture cells, in the absence of cycloheximide in order to encourage cryptic infection. Cryptic infection is uncommon in cells used in standard cell culture susceptibility techniques because *Chlamydia* in cycloheximide-paralyzed cells need not compete with the host cell for metabolites and hence are encouraged to replicate.

The *in vitro* method uses standard tissue culture cells, but without the addition of cycloheximide. Moreover, the chlamydiae are allowed to replicate for several days prior to the addition of at least one test agents. A "test agent" can be any compound to be evaluated as an antichlamydial agent for its ability to significantly reduce the presence of *Chlamydia* in living cells. For example, a test agent can include, but is not limited to, antibiotics, antimicrobial agents, antiparasitic agents, antimalarial agent, disulfide reducing agents and antimycobacterial agents. The test agent(s) is/are replaced when needed for the duration of the incubation time (days to weeks) to ensure that the test agent is present and has not been otherwise degraded. Antimicrobial agent(s) (test agent) is then added to the replicating cells. The antimicrobial agents/growth medium are periodically replaced for the duration of the incubation time, which is preferably weeks rather than days. Finally, the end point after the prolonged incubation time is the complete absence of chlamydial DNA, as determined by a

nucleic acid amplification technique, such as the
polymerase chain reaction (PCR) methodology. Standard
nucleic acid amplification techniques (such as PCR) are
used to ascertain the presence or absence of signal for
5 chlamydial DNA encoding MOMP or other unique *Chlamydia*
protein to determine whether the test agent or combination
of agents is/are effective in reducing *Chlamydia* infection.
The loss of signal (i.e., below the detectable level of the
nucleic acid amplification technique) in cells with
10 antibiotic(s) versus its presence in controls is an
indication of efficacy of the agent or combination of
agents against *Chlamydia*.

Accordingly, the susceptibility test of this invention
can be used to identify an agent or agents which are
15 effective against any particular species of *Chlamydia* and
can be used to identify agent(s) effective against the
cryptic form of the pathogen, i.e., is capable of
inhibiting or eliminating the cryptic form of the pathogen.
Agents that are effective against *Chlamydia*, as ascertained
20 by the susceptibility testing protocols described herein,
can be used as part of a therapy for the management of
Chlamydia infections. Suitable therapeutic protocols are
described in detail below, with a particular focus on
targeting agents toward specific stages of the chlamydial
25 life cycle.

The methods described herein are unique because they
evaluate the activity of antimicrobial agents in the
absence of cycloheximide which provides a more clinically
relevant intracellular milieu. For example, any energy-
30 dependent host cell membrane pumps which might move
antimicrobial agents in or out of the cell are inactivated
by the use of cycloheximide. The methods described herein
are unique because they utilize culture medium which has
previously been inactivated. The methods are also unique
35 because they measures the effect of a prolonged duration of

When PCR is the preferred methodology used to evaluate assay endpoint, the PCR method can be enhanced by the unique application of a reducing agent, such as dithiothreitol (DTT), in order to uncoat chlamydial EBs and hence allow exposure of the DNA. In other words, DTT permits the EB coating to rupture. By using an assay for DNA in which EBs are specifically uncoated, the susceptibility test endpoint assesses the presence or absence of EBs as well as the presence or absence of both replicating and nonreplicating RBs. Thus, this approach for chlamydial susceptibility testing allows quantitative antimicrobial susceptibility assays of single and combination agents in which the cumulative effect of the agent(s) on the complete eradication of all life phases is measured. Examples of results obtained with this *in vitro* method are described below.

In one embodiment, a suitable nucleic acid assay for identifying agents effective against the cryptic form of chlamydia comprises, in the presence of agent(s) to be tested, subjecting cultured cells to protease/reducing agent (e.g., dithiotreitol) and protease digestion or guanidine isothiocyanate (also known as guanidine thiocyanate) for a prescribed period of time; extracting DNA from the treated solution; exposing DNA to appropriate polymerase, dNTPs and primers for DNA amplification of MOMP or other protein of the *Chlamydia* species; and determining

5 primers are CHLMOMPDB2 and CHLMOMPCB2.

10 protease digestion; stopping protease activity; exposing
cells to appropriate heat-stable DNA polymerase, dNTPs and
labeled primers (e.g., 3'-biotin labeled, 5'-biotin
labeled) for amplification of DNA encoding MOMP of the
Chlamydia species; washing the cells; exposing the cells to
15 a reporter molecule (e.g., strepavidin-conjugated signal
enzyme); exposing the cells to an appropriate substrate for
the reporter molecule (e.g., conjugated enzyme); and
visualizing the amplified DNA encoding MOMP by visualizing
the product of the reaction.

25 appropriate polymerase, dNTPs and primers for DNA
amplification of nucleic acid encoding of a chlamydial
protein; exposing the cells to a reporter molecule enzyme;
exposing the cells to an appropriate substrate for the
reporter enzyme; and determining the presence of the
30 cryptic form of *Chlamydia* by visualizing the amplified DNA
encoding a chlamydial protein. Preferably the
amplification technique is PCR and the primers are
CHLMOMPDB2 and CHLMOMPCB2 of *Chlamydia pneumoniae*.

35.. identifying an agent which is effective against the cryptic

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form of *Chlamydia*. Accordingly, the method comprises treating cultured cells grown in the absence of cycloheximide, thought to be infected with *Chlamydia*, with a disulfide reducing agent; allowing the *Chlamydia* to replicate; adding a test agent; subjecting cultured cells to protease digestion; exposing cells to appropriate polymerase, dNTPs and primers for DNA amplification of a gene encoding chlamydial protein; exposing the cells to a reporter molecule enzyme; exposing the cells to an appropriate substrate for the reporter enzyme; and determining the presence of cryptic form of *Chlamydia* by visualizing the amplified DNA encoding a chlamydial protein, such as MOMP.

A detailed description of primers, PCR techniques and other methodologies useful for the present invention are provided in U.S. Patent Application Serial No. _____ entitled "Identification of Antigenic Peptide Sequences" (Attorney Docket No. VDB98-01), filed concurrently herewith; the entire teachings of this application are incorporated herein by reference.

B. In Vivo Methodology

In another aspect of the invention, the susceptibility test can be used to evaluate the status of a human or animal undergoing therapy for the management of *Chlamydia* infection. For example, a biological material is isolated from the human or animal undergoing combination therapy. The biological material is treated such that the *Chlamydia* is isolated therefrom. This chlamydial isolate is allowed to infect *Chlamydia* free cells. These infected cells are then exposed to the combination of agents being used in the individual undergoing combination therapy. Alternatively, the individual's serum containing the antimicrobial agents can be added to the infected cells as a "serum bactericidal test" for intracellular chlamydial infection.

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The *in vivo* method uses the murine model although other animals such as rats or rabbits can be used. In this method, mice (or any other animal) are inoculated intranasally with 2×10^5 chlamydial EBs per ml. The

5 inventors have confirmed the work of Yang and colleagues (15) in which intranasal inoculation of chlamydial EBs results in systemic dissemination and, in particular, causes infection of the spleen. The inventors have discovered that this systemic dissemination also results in

10 the presence of EBs in the blood of the mice. Therefore, infectivity can be measured by blood culture or by serum/whole blood PCR for chlamydial DNA. Systemic infection is also confirmed and monitored by the presence of elevated IgM and IgG antibody titers. After the

15 systemic murine infection has been established, antimicrobial agents are given to the mice. This is most easily done by adding the antibiotics to the drinking water. The effect of antichlamydial therapy is monitored by serum/whole blood PCR. When the serum/PCR assay

20 suggests eradication of chlamydiae from the bloodstream, the mice are sacrificed and PCR for chlamydial DNA is done on lung, heart, liver, and spleen homogenates. This method is unique because it measures the complete eradication of

25 all life forms of chlamydiae in known murine target organs for chlamydial infection. This *in vivo* susceptibility method has revealed, for example, that antimicrobial therapy with the triple agents, INH, metronidazole and penicillamine, can completely eradicate *C. pneumoniae* from

30 infected mice in four months. Moreover, following complete eradication of chlamydiae, multiple attempts to reinfect these cured mice via intranasal inoculation have proven unsuccessful. This suggests that effective therapy and complete eradication results in the development of

protective immunity, and that effective therapy is therefore a way to create effective immunity.

Performing PCR for chlamydial DNA on homogenates of other organ systems can be used to determine the effectiveness of particular antibiotic combinations in eradicating chlamydial infection in those organ systems. Establishment of prior chlamydial infection of those systems can be done by either biopsy or antibody-enhanced radiological imaging. Alternatively, prior infection can be determined statistically by performing PCR for chlamydial DNA on homogenates of the same organ systems in a similarly inoculated but untreated control population. Organ-specific susceptibility is determined by comparing rates of positive PCR assays in the control and treated populations.

An alternative or complementary method of determining the presence of cryptic chlamydial infections in an animal or cell culture is to expose the culture to chlamydia-stimulating compounds. Such compounds include (but are not limited to) cycloheximide, corticosteroids (such as prednisone) and other compounds which are known to stimulate reactivation of cryptic intracellular infections, and disulfide reducing agents (such as dithiotreitol) and other chemicals which cause EBs to turn into RBs. Once the cryptic forms have entered a more active phase, they can be detected using standard detection techniques such as visual detection of inclusion bodies, immunochemical detection of chlamydial antigen, or reverse transcriptase-PCR.

ANTICHLAMYDIAL THERAPY DIRECTED TOWARD THE INITIAL STAGE OF CHLAMYDIA INFECTION

A number of effective agents that are specifically directed toward the initial phase of chlamydial infection

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(i.e., transition of the chlamydial EB to an RB) have been identified. This growth phase, unlike that of the replicating chlamydial microorganism, which uses host cell energy, involves electrons and electron transfer proteins, as well as nitroreductases. Based upon this, it has been shown that the initial phase of *Chlamydia* infection is susceptible to the antimicrobial effects of nitroimidazoles, nitrofurans and other agents directed against anaerobic metabolism in bacteria.

Nitroimidazoles and nitrofurans are synthetic antimicrobial agents that are grouped together because both are nitro (NO_2^-) containing ringed structures and have similar antimicrobial effects. These effects require degradation of the agent within the microbial cell such that electrophilic radicals are formed. These reactive electrophilic intermediates then damage nucleophilic protein sites including ribosomes, DNA and RNA. Nitroimidazoles and nitrofurans currently are not considered to possess antimicrobial activity against members of the *Chlamydia* species. This lack of antimicrobial activity, however, is due to the fact that conventional susceptibility testing methods only test for effect on the replicating form of *Chlamydia* species.

Examples of suitable nitroimidazoles include, but are not limited to, metronidazole, tinidazole, bamnidazole, benznidazole, flunidazole, ipronidazole, misonidazole, moxnidazole, ronidazole, sulnidazole, and their metabolites, analogs and derivatives thereof.

Metronidazole is most preferred. Examples of nitrofurans that can be used include, but are not limited to, nitrofurantoin, nitrofurazone, nifurtimox, nifuratel, nifuradene, nifurdazil, nifurpirinol, nifuratrone, furazolidone, and their metabolites, analogs and

[illegible]

Cells to be treated can already be cryptically infected or they can be subjected to stringent metabolic conditions which cause or induce the replicating phase to enter the cryptic phase. Such stringent conditions can include
20 changing environmental/culturing conditions in the instance where the infected cells are exposed to γ -interferon; or by exposing cells to conventional antimicrobial agents (such as macrolides and tetracyclines) which induce this cryptic phase of chlamydial infection in human host cells.

A unique class of antichlamydial agents that is effective against the replicating phase of *Chlamydia* (and possibly against some stages of the cryptic stage) have been identified using the susceptibility tests described herein. This novel class of agents comprises ethambutol and isonicotinic acid congeners which include isoniazid

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(INH), isonicotinic acid (also known as niacin), nicotinic acid, pyrazinamide, ethionamide, and aconiazide; where INH is most preferred. Although these are currently considered effective only for mycobacterial infections, due in part to
5 currently available susceptibility testing methodologies, it has been discovered that these agents, in combination with other antibiotics, are particularly effective against *Chlamydia*. It is believed that the isonicotinic acid congeners target the constitutive production of catalase
10 and peroxidase, which is a characteristic of microorganisms, such as mycobacteria, that infect monocytes and macrophages. *Chlamydia* can also successfully infect monocytes and macrophages.

Using INH to eradicate *Chlamydia* from macrophages and
15 monocytes subsequently assists these cells in their role of fighting infection. However, these agents appear to be less effective, *in vitro*, against the cryptic phase. Thus, ethambutol, INH and other isonicotinic acid congeners ideally should be used in combination with agents that
20 target other phases of the chlamydial life cycle. These isonicotinic acid congeners are nevertheless excellent agents for the long term therapy of chronic/systemic chlamydial infection generally, and in particular to chlamydial infection of endothelial and smooth muscle cells
25 in human blood vessels.

INH and its congeners can be used to clear infection from monocytes and/or macrophages. When monocytes and macrophages are infected by *Chlamydia*, they become debilitated and cannot properly or effectively fight
30 infection. It is believed that, if the chlamydial infection, *per se*, is cleared from these cells, then the monocytes and macrophages can resume their critical roles fighting chlamydial or other infection(s). Thus, patient

5 compromised by a *Chlamydia* infection and, in turn, comprise
treating the infection in other sites. Such compromised
macrophages or monocytes can be activated by treating the
chlamydia infection by contacting the infected macrophages
and/or monocytes with an antichlamydial agent.

10 THERAPY DIRECTED TOWARD ELEMENTARY BODIES OF *CHLAMYDIA*

As discussed above, it has been discovered that adverse conditions, such as limited nutrients, antimicrobial agents, and the host immune response, produce a stringent response in *Chlamydia*. Such adverse conditions are known to induce stringent responses in other microorganisms (C.W. Stratton, In: *Antibiotics in Laboratory Medicine*, Fourth Edition. Lorian V (ed) Williams & Wilkins, Baltimore, pp 579-603 (1996)) and not surprisingly induce a stringent response in *Chlamydia*. This stringent response in *Chlamydia* alters the morphological state of the intracellular microorganism and creates a dormant form, the intracellular EB, which then can cryptically persist until its developmental cycle is reactivated. Conversely, the host cell may lyse and allow the EBs to reach the extracellular milieu. Thus, it is necessary to utilize a combination of agents directed toward the various life stages of *Chlamydia* and, in particular, against the elementary body for successful management of infection.

During the unique chlamydial life cycle, it is known
30 that metabolically-inactive spore-like EBs are released
into the extracellular milieu. Although these released EBs
are infectious, they may not immediately infect nearby

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susceptible host cells until appropriate conditions for EB infectivity are present. The result of this delay in infection is the extracellular accumulation of metabolically-inactive, yet infectious, EBs. This produces a second type of chlamydial persistence referred to herein as EB "tissue/blood load". This term is similar in concept to HIV load and is defined herein as the number of infectious EBs that reside in the extracellular milieu. Direct microscopic visualization techniques, tissue cell cultures, and polymerase chain reaction test methods have demonstrated that infectious EBs are frequently found in the blood of apparently healthy humans and animals. This phenomenon is clearly of great clinical importance in chlamydial infections as these metabolically-inactive EBs escape the action of current antichlamydial therapy which is directed only against the replicating intracellular forms of *Chlamydia*. The presence of infectious extracellular EBs after the completion of short term, anti-replicating phase therapy for chlamydial infections has been shown to result in infection relapse. Thus, the duration and nature of antichlamydial therapy required for management of chlamydial infections is, in part, dictated by the extracellular load of EBs. For purposes of this invention, short term therapy can be approximately two to three weeks; long term therapy in contrast is for multiple months.

As described in previous sections, it is also believed that persistence of chlamydial infections, in part, may be due to the presence of the cryptic form of *Chlamydia* within the cells. This cryptic intracellular chlamydial form apparently can be activated by certain host factors such as cortisone (Yang et al., *Infection and Immunity*, 39:655-658 (1983); and Malinverni et al., *The Journal of Infectious*

Diseases, 172:593-594 (1995)). Antichlamydial therapy for chronic *Chlamydia* infections must be continued until any intracellular EBs or other intracellular cryptic forms have been activated and extracellular EBs have infected host
5 cells. This reactivation/reinfection by chlamydial EBs clearly is undesirable as it prolongs the therapy of chlamydial infections, as well as increases the opportunity for antimicrobial resistance to occur.

Physiochemical agents have been identified that can
10 inactivate chlamydial EBs in their respective hosts by reducing disulfide bonds which maintain the integrity of the outer membrane proteins of the EBs. For *Chlamydia*, disruption of the outer membrane proteins of EBs thereby initiates the transition of the EB form to the RB form.
15 When this occurs in the acellular milieu where there is no available energy source, the nascent RB perishes or falls victim to the immune system. Thus, disulfide reducing agents that can interfere with this process are suitable as compounds for eliminating EBs.

20 One such class of disulfide reducing agents are thiol-disulfide exchange agents. Examples of these include, but are not limited to, 2,3-dimercaptosuccinic acid (DMSA; also referred to herein as "succimer"); D,L,- β,β -dimethylcysteine (also known as penicillamine); β -lactam
25 (e.g., penicillins, penicillin G, ampicillin and amoxicillin, which produce penicillamine as a degradation product), cycloserine, dithiotreitol, mercaptoethylamine (e.g., mesna, cysteamine, dimercaptol), N-acetylcysteine, tiopronin, and glutathione. A particularly effective
30 extracellular antichlamydial agent within this class is DMSA which is a chelating agent having four ionizable hydrogens and two highly charged carboxyl groups which prevent its relative passage through human cell membranes.

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DMSA thus remains in the extracellular fluid where it can readily encounter extracellular EBs. The two thiol (sulfhydryl) groups on the succimer molecule (DMSA) are able to reduce disulfide bonds in the MOMP of EBs located in the extracellular milieu.

Penicillamine can also be used as a disulfide reducing agent to eliminate chlamydial EBs. However, the use of penicillamine may cause undesirable side effects. Thus, as an alternative, those β -lactam agents which are metabolized or otherwise converted to penicillamine-like agents *in vivo* (i.e., these agents possess a reducing group) can be orally administered to the human or animal as a means of providing a controlled release of penicillamine derivatives, by acid hydrolysis of the penicillin, under physiologic conditions. The *in vivo* production of penicillamine from the degradation of penicillins undoubtedly accounts for the known *in vitro* ability of penicillins to reduce or prevent the development of infectious chlamydial EBs in cell cultures.

CURRENTLY RECOGNIZED AGENTS ACTIVE AGAINST *CHLAMYDIA* REPLICATION

As chlamydial RBs transform into EBs, they begin to utilize active transcription of chlamydial DNA and translation of the resulting mRNA. As such, these forms of *Chlamydia* are susceptible to currently used antimicrobial agents. The antichlamydial effectiveness of these agents can be significantly improved by using them in combination with other agents directed at different stages of *Chlamydia* life cycle, as discussed herein.

Classes of suitable antimicrobial agents include, but are not limited to, rifamycins (also known as ansamacrolides), quinolones, fluoroquinolones,

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Table 5

Agents Effective Against the Replicating
Phase of *Chlamydia*

Drug Class	Examples	Preferred
Quinolones/ Fluoroquinolones	Ofloxacin Levofloxacin Trovafoxacin Sparfloxacin Norfloxacin Lomefloxacin Cinoxacin Enoxacin Nalidixic Acid Fleroxacin Ciprofloxacin	Levofloxacin
Sulfonamides	Sulfamethoxazole	Sulfamethoxazole/ Trimethoprim
Azalides	Azithromycin	Azithromycin
Macrolides	Erythromycin Clarithromycin	Clarithromycin
Lincosamides	Lincomycin Clindamycin	
Tetracyclines	Tetracycline Doxycycline Minocycline Methacycline Oxytetracycline	Minocycline
Rifamycins (Ansamacrolides)	Rifampin Rifabutin	Rifampin

All members of the *Chlamydia* species, including *C. pneumoniae*, are considered to be inhibited, and some killed, by the use of a single agent selected from currently used antimicrobial agents such as those described above. However, using the new susceptibility test, the inventors have found complete eradication of *Chlamydia* cannot be achieved by the use of any one of these agents alone because none are efficacious against all phases of

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the *Chlamydia* life cycle and appear to induce a stringent response in *Chlamydia* causing the replicating phase to transform into cryptic forms. This results in a persistent infection *in vivo* or *in vitro* that can be demonstrated by

5 PCR techniques which assess the presence or absence of chlamydial DNA. Nevertheless, one or more of these currently used agents, or a new agent directed against the replicating phase of *Chlamydia*, should be included as one of the chlamydial agents in a combination therapy in order

10 to slow or halt the transition of the EB to the RB as well as to inhibit chlamydial replication.

DISEASES ASSOCIATED WITH CHLAMYDIAL INFECTION

An association has been discovered between chronic *Chlamydia* infection of body fluids and/or tissues with

15 several disease syndromes of previously unknown etiology in humans which respond to unique antichlamydial regimens described herein. To date, these diseases include Multiple Sclerosis (MS), Rheumatoid Arthritis (RA), Inflammatory Bowel Disease (IBD), Interstitial Cystitis (IC),

20 Fibromyalgia (FM), Autonomic nervous dysfunction (AND, neural-mediated hypotension); Pyoderma Gangrenosum (PG), Chronic Fatigue (CF) and Chronic Fatigue Syndrome (CFS). Other diseases are under investigation. Correlation between *Chlamydia* infection and these diseases has only

25 recently been established as a result of the diagnostic methodologies and combination therapies described herein.

Based on this evidence, published evidence of an association between atherosclerosis and *Chlamydia* (Grupta et al., *Circulation* 96:404-407 (1997)), and an

30 understanding of the impact *Chlamydia* infections have on infected cells and the immune systems, the inventors have discovered a connection between *Chlamydia* and a broad set of inflammatory, autoimmune, and immune deficiency diseases. Thus, the invention describes methods for

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diagnosing and/or treating disease associated with *Chlamydia* infection, such as autoimmune diseases, inflammatory diseases and diseases that occur in immunocompromised individuals by diagnosing and/or treating the *Chlamydia* infection in an individual in need thereof, using any of the assays or therapies described herein.

Progress of the treatment can be evaluated serologically, to determine the presence or absence of *Chlamydia* using for example the diagnostic methods provided herein, and this value can be compared to serological values taken earlier in the therapy. Physical improvement in the conditions and symptoms typically associated with the disease to be treated should also be evaluated. Based upon these evaluating factors, the physician can maintain or modify the antichlamydial therapy accordingly. For example, the physician may change an agent due to adverse side-effects caused by the agent, ineffectiveness of the agent, or for other reason. When antibody titers rise during treatment then alternate compounds should be substituted in order to achieve the lower antibody titers that demonstrate specific susceptibility of the *Chlamydia* to the new regimen. A replacement or substitution of one agent with another agent that is effective against the same life stage of *Chlamydia* is desirable.

The therapies described herein can thus be used for the treatment of acute and chronic immune and autoimmune diseases when demonstrated to have a *Chlamydia* load by the diagnostic procedures described herein which include, but are not limited to, chronic hepatitis, systemic lupus erythematosus, arthritis, thyroidosis, scleroderma, diabetes mellitus, Graves' disease, Beschets' disease and graft versus host disease (graft rejection). The therapies of this invention can also be used to treat any disorders in which a chlamydial species is a factor or co-factor.

Thus, the present invention can be used to treat a range of disorders in addition to the above immune and autoimmune diseases when demonstrated to be associated with Chlamydial infection by the diagnostic procedures described herein; for example, various infections, many of which produce inflammation as primary or secondary symptoms, including, but not limited to, sepsis syndrome, cachexia, circulatory collapse and shock resulting from acute or chronic bacterial infection, acute and chronic parasitic and/or infectious diseases from bacterial, viral or fungal sources, such as a HIV, AIDS (including symptoms of cachexia, autoimmune disorders, AIDS dementia complex and infections) can be treated, as well as Wegners Granulomatosis.

Among the various inflammatory diseases, there are certain features of the inflammatory process that are generally agreed to be characteristic. These include fenestration of the microvasculature, leakage of the elements of blood into the interstitial spaces, and migration of leukocytes into the inflamed tissue. On a macroscopic level, this is usually accompanied by the familiar clinical signs of erythema, edema, tenderness (hyperalgesia), and pain. Inflammatory diseases, such as chronic inflammatory pathologies and vascular inflammatory pathologies, including chronic inflammatory pathologies such as aneurysms, hemorrhoids, sarcoidosis, chronic inflammatory bowel disease, ulcerative colitis, and Crohn's disease and vascular inflammatory pathologies, such as, but not limited to, disseminated intravascular coagulation, atherosclerosis, and Kawasaki's pathology are also suitable for treatment by methods described herein. The invention can also be used to treat inflammatory diseases such as coronary artery disease, hypertension, stroke, asthma, chronic hepatitis, multiple sclerosis, peripheral neuropathy, chronic or recurrent sore throat, laryngitis,

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Table 1. Demographic characteristics of the study population	
Age (years)	65.0 ± 1.5
Gender	
Male	50.0
Female	50.0
Education (years)	12.0 ± 1.0
Income (USD/month)	1,200.0 ± 200.0
Marital status	
Married	60.0
Single	40.0
Divorced	10.0
Widowed	10.0
Health status	
Good	70.0
Fair	20.0
Poor	10.0
Smoking status	
Smoker	30.0
Non-smoker	70.0
Alcohol consumption	
Drinker	20.0
Non-drinker	80.0
Comorbidities	
Hypertension	40.0
Diabetes	20.0
Heart disease	10.0
Stroke	10.0
Chronic kidney disease	10.0
Chronic liver disease	10.0
Chronic respiratory disease	10.0
Chronic pain	10.0
Chronic mental health	10.0
Chronic infection	10.0
Chronic autoimmune	10.0
Chronic cancer	10.0
Chronic other	10.0
Medication use	
Antihypertensives	40.0
Antidiabetics	20.0
Cardiovascular	10.0
Neurological	10.0
Respiratory	10.0
Painkillers	10.0
Mental health	10.0
Antibiotics	10.0
Other	10.0
Healthcare utilization	
Primary care visits	1.0 ± 0.5
Specialty care visits	0.5 ± 0.2
Hospitalizations	0.2 ± 0.1
Emergency department visits	0.1 ± 0.1
Health insurance	
Medicaid	60.0
Medicare	40.0
Private	10.0
Uninsured	10.0
Health literacy	
High	70.0
Medium	20.0
Low	10.0
Health beliefs	
Preventive care	60.0
Self-management	50.0
Health communication	40.0
Health equity	30.0
Health justice	20.0
Health care	10.0
Health system	10.0
Health policy	10.0
Health research	10.0
Health education	10.0
Health promotion	10.0
Health behavior	10.0
Health status	10.0
Health outcomes	10.0
Health equity	10.0
Health justice	10.0
Health care	10.0
Health system	10.0
Health policy	10.0
Health research	10.0
Health education	10.0
Health promotion	10.0
Health behavior	10.0
Health status	10.0
Health outcomes	10.0
Health equity	10.0
Health justice	10.0
Health care	10.0
Health system	10.0
Health policy	10.0
Health research	10.0
Health education	10.0
Health promotion	10.0
Health behavior	10.0
Health status	10.0
Health outcomes	10.0
Health equity	10.0
Health justice	10.0
Health care	10.0
Health system	10.0
Health policy	10.0
Health research	10.0
Health education	10.0
Health promotion	10.0
Health behavior	10.0
Health status	10.0
Health outcomes	10.0
Health equity	10.0
Health justice	10.0
Health care	10.0
Health system	10.0
Health policy	10.0
Health research	10.0
Health education	10.0
Health promotion	10.0
Health behavior	10.0
Health status	10.0
Health outcomes	10.0
Health equity	10.0
Health justice	10.0
Health care	10.0
Health system	10.0
Health policy	10.0
Health research	10.0
Health education	10.0
Health promotion	10.0
Health behavior	10.0
Health status	10.0
Health outcomes	10.0
Health equity	10.0
Health justice	10.0
Health care	10.0
Health system	10.0
Health policy	10.0
Health research	10.0
Health education	10.0
Health promotion	10.0
Health behavior	10.0
Health status	10.0
Health outcomes	10.0
Health equity	10.0
Health justice	10.0
Health care	10.0
Health system	10.0
Health policy	10.0
Health research	10.0
Health education	10.0
Health promotion	10.0
Health behavior	10.0
Health status	10.0
Health outcomes	10.0
Health equity	10.0
Health justice	10.0
Health care	10.0
Health system	10.0
Health policy	10.0
Health research	10.0
Health education	10.0
Health promotion	10.0
Health behavior	10.0
Health status	10.0
Health outcomes	

- 5 Treatable disorders when associated with *Chlamydia*
infection also include, but are not limited to,
neurodegenerative diseases, including, but not limited to,
demyelinating diseases, such as multiple sclerosis and
acute transverse myelitis; extrapyramidal and cerebellar
10 disorders, such as lesions of the corticospinal system;
disorders of the basal ganglia or cerebellar disorders;
hyperkinetic movement disorders such as Huntington's Chorea
and senile chorea; drug-induced movement disorders, such as
those induced by drugs which block CNS dopamine receptors;
15 hypokinetic movement disorders, such as Parkinson's
disease; Progressive supranucleo palsy; Cerebellar and
Spinocerebellar Disorders, such as astructural lesions of
the cerebellum; spinocerebellar degenerations (spinal
ataxia, Friedreich's ataxia, cerebellar cortical
20 degenerations, multiple systems degenerations (Mencel,
Dejerine-Thomas, Shi-Drager, and Machado Joseph)); and
systemic disorders (Refsum's disease, abetalipoproteinemia,
ataxia, telangiectasia, and mitochondrial multi-system
disorder); demyelinating core disorders, such as multiple
25 sclerosis, acute transverse myelitis; disorders of the
motor unit, such as neurogenic muscular atrophies (anterior
horn cell degeneration, such as amyotrophic lateral
sclerosis, infantile spinal muscular atrophy and juvenile
spinal muscular atrophy); Alzheimer's disease; Down's
30 Syndrome in middle age; Diffuse Lewy body disease; Senile
Dementia of Lewy body type; Wernicke-Korsakoff syndrome;
chronic alcoholism; Creutzfeldt-Jakob disease; Subacute
sclerosing panencephalitis, Hallerorden-Spatz disease; and
Dementia pugilistica, or any subset thereof.

It is also recognized that malignant pathologies involving tumors or other malignancies, such as, but not limited to leukemias (acute, chronic myelocytic, chronic lymphocytic and/or myelodysplastic syndrome); lymphomas (Hodgkin's and non-Hodgkin's lymphomas, such as malignant lymphomas (Burkitt's lymphoma or Mycosis fungoides)); carcinomas (such as colon carcinoma) and metastases thereof; cancer-related angiogenesis; infantile hemangiomas; alcohol-induced hepatitis. Ocular neovascularization, psoriasis, duodenal ulcers, angiogenesis of the female reproductive tract, can also be treated when demonstrated by the diagnostic procedures described herein to be associated with Chlamydial infection.

An immunocompromised individual is generally defined as a person who exhibits an attenuated or reduced ability to mount a normal cellular or humoral defense to challenge by infectious agents, e.g., viruses, bacterial, fungi and protozoa. Persons considered immunocompromised include malnourished patients, patients undergoing surgery and bone marrow transplants, patients undergoing chemotherapy or radiotherapy, neutropenic patients, HIV-infected patients, trauma patients, burn patients, patients with chronic or resistant infections such as those resulting from myelodysplastic syndrome, and the elderly, all of who may have weakened immune systems. A protein malnourished individual is generally defined as a person who has a serum albumin level of less than about 3.2 grams per deciliter (g/dl) and/or unintentional weight loss greater than 10% of usual body weight.

The course of therapy, serological results and clinical improvements from compassionate antichlamydial therapy in patients diagnosed with the diseases indicated were observed and are reported in Example 5. The data provides evidence to establish that treatment of *Chlamydia* infection

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results in the serological and physical improvement of a disease state in the patient undergoing combination therapy. These observations were consistent among a variety of different diseases which fall within a
5 generalized disease class.

OTHER DISEASES OF UNKNOWN ETIOLOGY WITH NEW EVIDENCE FOR A CHLAMYDIA PNEUMONIAE ETIOLOGY

Both *C. trachomatis* and *C. psittaci* exhibit a protean disease complex dependent on different serovars. One known
10 basis for this diversity to date is the amino acid sequence of the MOMP. Fig. 1 shows a sequence alignment of various *Chlamydia* MOMPs. Note that the size and sequence are relatively homologous except for the four variable regions that are responsible for the serovar (serotype) basis of
15 classification. Further, it has been discovered that *C. pneumoniae* infects blood vessel endothelial cells from which EBs are released in the blood stream. In addition, macrophages are known targets for *C. pneumoniae* and may serve as reservoirs and provide an additional mechanism of
20 transmission. *C. pneumoniae* is thus able to spread throughout the human body, establishing infection in multiple sites and in multiple organ systems. Infected sites may exist for an extended period without inducing symptoms that are noticed by the patient or by an examining
25 physician. Sequence variability of MOMPs or other chlamydial antigens may provide a basis for organ specificity while other chlamydial proteins, such as the 60K and 70K heat shock proteins or LPS, may influence immune response.

30 *C. psittaci* and *C. pecorum* are known to cause a host of infections in economically significant animals. Thus, the teachings of this invention are relevant to animals. Throughout this application and for purposes of this invention, "patient" is intended to embrace both humans and

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animals. Virtually all rabbits and mice tested to date have PCR signals for *C. pneumoniae*. They can be used as appropriate animal models for treatment using specific combination antibiotics to improve therapy. (Banks et al.,
5 *Amer. J. of Obstetrics and Gynecology* 138(7Pt2):952-956 (1980)); (Moazed et al., *Am. J. Pathol.* 148(2):667-676 (1996)); (Masson et al., *Antimicrob. Agents Chemother.* 39(9):1959-1964 (1995)); (Patton et al., *Antimicrob. Agents Chemother.* 37(1):8-13 (1993)); (Stephens et al., *Infect.*
10 *Immun.* 35(2):680-684 (1982)); and (Fong et al., *J. Clin. Microbiol* 35(1):48-52 (1997)).

Coupled with these developments are the recently developed rabbit models of coronary artery disease, where rabbits exposed to *C. pneumoniae* subsequently develop
15 arterial plaques similar to humans (Fong et al., *J. Clin. Microbiol.* 35:48-52 (1997)). Most recently, a study at St. George's Hospital in London found that roughly 3/4 of 213 heart attack victims have significant levels of antibodies to *C. pneumoniae* antibody and that those that have such
20 antibodies achieve significantly lower rates of further adverse cardiac events when treated with antibiotics (Gupta et al., *Circulation* 95:404-407 (1997)). Taken together, these three pieces of evidence (the bacteria found in diseased tissue, inoculation with the bacteria causes
25 diseases, and treating for the bacteria mitigates disease) make a case for a causal connection.

ADJUNCT AGENTS USED IN CONJUNCTION WITH THE COMBINATION THERAPY

In addition to the combination therapies discussed
30 above, other compounds can be co-administered to an individual undergoing antichlamydial therapy for the management of chronic/systemic infection. For example, it may be desirable to include one or a combination of anti-inflammatory agents and/or immunosuppressive agents to

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amelioriate side-effects that may arise in response to a particular antichlamydial agent, e.g., Herxheimer reactions. Initial loading with an anti-inflammatory steroid can be introduced to minimize side-effects of the antichlamydial therapy in those patients in which clinical judgment suggests the possibility of serious inflammatory sequelae.

Suitable anti-inflammatory agents (steroidal and nonsteroidal agents) include, but are not limited to, Prednisone, Cortisone, Hydrocortisone and Naproxin. Preferably the anti-inflammatory agent is a steroidal agent, such as Prednisone. The amount and frequency of administration of these adjunct compounds will depend upon patient health, age, clinical status and other factors readily apparent to the medical professional.

Vitamin C (2 gms bid) has also been introduced based on the report that Vitamin C (ascorbic acid) at moderate intracellular concentrations stimulates replication of *C. trachomatis* (Wang et al., *J. Clin. Micro.* 30:2551-2554 (1992)) as well as its potential effect on biofilm charge and infectivity of the bacterium and specifically the EB (Hancock, R.E.W., *Annual Review in Microbiology*, 38:237-264 (1984)).

MODES OF ADMINISTRATION

Based upon the ability of the combination therapy of this invention to improve both the serological and physical status of a patient undergoing treatment, pharmaceutical compositions or preparations can be made comprising at least two different agents chosen from the following groups: a) at least one agent effective against elementary body phase of chlamydial life cycle (e.g., disulfide reducing agents); b) at least one agent effective against replicating phase of chlamydial life cycle (e.g., antimycobacterial agents); and c) at least one agent

effective against cryptic phase of chlamydial life cycle (e.g., anerobic bactericidal agents). As discussed in greater detail below, the agents can be formulated in a physiologically acceptable vehicle in a form which will be dependent upon the method in which it is administered.

In another aspect, the invention pertains to a combination of agents comprising at least two agents, each of which is effective against a different phase of the chlamydial life cycle, as previously discussed. The combination of antichlamydial can be used in the management of chlamydial infection or prophylaxis thereof to prevent recurrent infection. The combination of agents can be in the form of an admixture, as a pack (discussed in detail below) or individually, and/or by virtue of the instruction to produce such a combination. It should be understood that combination therapy can comprise multiple agents that are effective within a particular phase of the chlamydial life cycle. The combination of antichlamydial agents can further comprise immunosuppressants, anti-inflammatory agents, vitamin C and combinations thereof.

In a preferred embodiment, if only one antichlamydial agent is elected to be used in an asymptomatic patient to reduce/prevent chronic infection, this agent is a reducing agent, such as penicillamine.

The novel therapeutic methods described herein can be used to ameliorate conditions/symptoms associated with the disease states described above, when the disease is onset or aggravated by infection by *Chlamydia*. The agents of this invention can be administered to animals including, but not limited to, fish, amphibians, reptiles, avians and mammals including humans. Compounds and agents described herein can be administered to an individual using standard methods and modes which are typically routine for the disease state.

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membranes, such as sialastic membranes or fibers. For example, the agent can be injected into the joints.

In a specific embodiment when it is desirable to direct the drug to the central nervous system, techniques which
5 can opportunistically open the blood brain barrier for a time adequate to deliver the drug there through can be used. For example, a composition of 5% mannitose and water can be used. In another embodiment, the agents can be delivered to a fetus through the placenta since many of the
10 agents are small enough to pass through the placental barrier.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically (or prophylactically) effective amount of the agent, and a
15 pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The carrier and composition can be sterile. The formulation should suit the mode of
20 administration.

Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions (e.g., NaCl), alcohols, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as
25 lactose, amylose or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxymethylcellulose, polyvinyl pyrrolidone, etc. The pharmaceutical preparations can be sterilized and if desired, mixed with auxiliary agents, e.g., lubricants,
30 preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds.

The composition, if desired, can also contain minor
35 amounts of wetting or emulsifying agents, or pH buffering

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aerosols, etc., which are, if desired, sterilized or mixed with auxiliary agents, e.g., preservatives, stabilizers, wetting agents, buffers or salts for influencing osmotic pressure, etc. The drug may be incorporated into a cosmetic formulation. For topical application, also suitable are sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier material, is packaged in a squeeze bottle or in admixture with a pressurized volatile, normally gaseous propellant, e.g., pressurized air.

Agents described herein can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of agents which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such

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container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use of sale
5 for human administration. The pack or kit can be labeled with information regarding mode of administration, sequence of drug administration (e.g., separately, sequentially or concurrently), or the like. The pack or kit may also include means for reminding the patient to take the
10 therapy. The pack or kit can be a single unit dosage of the combination therapy or it can be a plurality of unit dosages. In particular, the agents can be separated, mixed together in any combination, present in a single vial or tablet. Agents assembled in a blister pack or other
15 dispensing means is preferred. For the purpose of this invention, unit dosage is intended to mean a dosage that is dependent on the individual pharmacodynamics of each agent and administered in FDA approved dosages in standard time courses.

20 DIAGNOSTIC REAGENTS

The invention also provides a diagnostic reagent pack or kit comprising one or more containers filled with one or more of the ingredients used in the assays of the invention. Optionally associated with such container(s)
25 can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of diagnostic products, which notice reflects approval by the agency of manufacture, use of sale for human administration. The pack or kit can be labeled with
30 information regarding mode of administration, sequence of execution (e.g., separately, sequentially or concurrently), or the like. The pack or kit can be a single unit assay or it can be a plurality of unit assays. In particular, the agents can be separated, mixed together in any combination,

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present in a single fial or tablet. For the purpose of this invention, unit assays is intended to mean materials sufficient to perform only a single assay.

The invention will be further illustrated by the following non-limiting examples of diagnostic and therapeutic methods. All percentages are by weight unless otherwise specified.

EXAMPLES

EXAMPLE 1

10 POLYMERASE CHAIN REACTION (PCR) FOR THE FULL LENGTH MOMP GENE OF *C. PNEUMONIAE* AND OTHER SPECIES OF *CHLAMYDIA* (DIAGNOSTIC)

a. Solution PCR

Serum, blood or tissue samples were pre-incubated in the presence of 10 μ M dithiothreitol at room temperature for 2 hours to reduce the disulfide bonds and facilitate release of the outer shell of the elementary bodies. CSF and other body fluids are also suitable for use as described. Other suitable reducing agents for use in this step include, but are not limited to, succimer and glutathione (e.g., including, but not limited to, glutathione esters, other analogs and derivatives). The failure to include a reducing agent initially may result in a negative PCR signal following the protease digestion step. Appropriate concentrations of these reducing agents can be readily determined by the skilled artisan without undue experimentation using the 10 μ M concentration of dithiothreitol as a guideline. Alternatively, guanidine isothiocyanate may be substituted for the disulfide reduction/protease step. Table 6 shows the effect of various reducing agents on susceptibility of EBs to

[illegible]

Table 6

Effect of various reducing agents on susceptibility of EBs to proteinase K digestion in order to allow DNA extraction for PCR amplification.

Reducing Agent	Concen- tration	PCR Signal ^a	Reducing Agent	Concen- tration	PCR Signal ^a
Dithiothreitol	10mM	+	2,3-Dimercapto-1- Propone-sulfide acid	10mM	-
	1mM	+		1mM	-
	100µM	+		100µM	+
	10µM	+		10µM	-
	1µM	+		1µM	-
Succimer	10mM	-	Meso-2,2'-Dimercapto adipic acid	10mM	+
	1mM	+		1mM	+
	100µM	+		100µM	+
	10µM	+		10µM	+
	1µM	-		1µM	-
DL-Penicillamine	10mM	-	Glutathione	10mM	-
	1mM	-		1mM	wk+
	100µM	+		100µM	-
	10µM	-		10µM	+/-
	1µM	-		1µM	+/-
D-Penicillamine disulfide	10mM	+	Control	0	-
	1mM	-			
	100µM	-			
	10µM	-			
	1µM	-			

a. All assays performed on control serum #1154, which on repeated assay without reducing agents, yields a negative PCR signal for the 1.2kB MOMP gene of *C. pneumoniae*. Analysis on agarose gel with ethidium bromide visualization under UV light.

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Serum, blood, or tissue samples are lysed overnight at 37°C in the presence of SDS which inhibits DNases and proteinase K which digests protein (i.e., 2 x cell lysis buffer: 1% SDS, 0.2 M NaCl, 10 mM EDTA, 20 mM Tris-KCl, pH 7.5 plus proteinase K to a final concentration of 20 mg/ml). Following digestion, the lysate is extracted x 1 with phenol followed by chloroform extraction x 2. DNA is precipitated from the final 'aqueous phase by the addition of 1/10 volume Na acetate (3 M) and 2-2.5 volume of cold ethanol. The DNA is pelleted by centrifugation and the DNA is resuspended in 10-20 ml water with PCR amplification performed in the same microtube. The entire gene of MOMP (1.2 kb) is amplified using the CHLMOMPDB2 coding strand primer (5'-ATGAAAAAAC TCTTAAAGTC GCGGTTATTA TCCGCCGC; SEQ ID NO. 41) and the CHLMOMPCB2 complimentary strand primer (5'-TTAGAATCTG AACTGACCAG ATACGTGAGC AGCTCTCTCG; SEQ ID NO. 42). Alternatively, shortened primers can be used by making suitable modifications in the primer:DNA hybridization temperature for PCR detection only. The appropriate primer selection, however, may result in the absence of signal if an unknown strain with mutations in one or both primer binding regions is present. The frequency of positive signals using the preferred primers which amplify the full length MOMP gene suggests that mutations in these regions of *C. pneumoniae* is rare. Standard conditions for this gene product in a 50- μ l volume is 35 cycles with 1 second ramp times between steps of 94°C for 1 minute, 58°C for 2 minutes and 74°C for 3 minutes. The PCR reaction used 0.1 mM of each primer in Vent buffer with 200 mM of each dNTP, and 1.0 U Vent DNA polymerase. Amplified DNA is separated and identified by electrophoresis in 1.2% agarose or 6% polyacrylamide gels run in the TBE buffer (88 mM Tris-borate, 89 mM boric acid, 2 mM EDTA) at 120 volts for 1 hour. DNA bands are

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substrate yielding an insoluble product which can then be visualized by standard light microscopy.

Alternatively, other specific DNA sequences, including subsections of the full MOMP gene (e.g., subsections including gene sequences for the peptides in Figure 4) can be used, although the above-described sequence is the preferred embodiment since the large product produced (1.2 kb) prevents diffusion that may be encountered with smaller DNA amplifications. Similarly, other detection labels can

be incorporated (i.e., fluorescein, for example) at the 5'-end or dioxigenin & UTP can be incorporated within the amplified DNA. Alternatively to labeling the product, specific hybridization probes to constant regions of the amplified DNA can be used to identify an amplified product. This latter method has particular utility for the construction of automated laboratory equipment for solution-based PCR. For example, strepavidin-coated ELISA plates can be used to capture one or both strands of a biotin 5'-labeled DNA with detection by fluorescence of a fluorescein or other incorporated fluorophore detection probe.

EXAMPLE 2

ENZYME LINKED IMMUNO SORBENT ASSAY (ELISA; DIAGNOSTIC)

a. Recombinant MOMP-Based ELISA

The full length MOMP gene of *C. pneumoniae* was directionally cloned into the pET expression plasmid at the NCOI and NOTI restriction sites using primers to introduce these unique restriction sites into the MOMP ends. Primer sequences are as follows:

CPOMPDNCO (Coding strand): 5'-AGCTTACCAT GGCTAAAAAA
CTCTTAAAGT CGGCGTTATT ATCCG-3' (SEQ ID NO. 43)

Table 1. Demographic characteristics of the study population	
Age (years)	Mean (SD)
18-24	20.5 (2.5)
25-34	29.5 (4.5)
35-44	39.5 (5.5)
45-54	49.5 (6.5)
55-64	59.5 (7.5)
65-74	69.5 (8.5)
75-84	79.5 (9.5)
85-94	89.5 (10.5)
95-104	99.5 (11.5)
105-114	109.5 (12.5)
115-124	119.5 (13.5)
125-134	129.5 (14.5)
135-144	139.5 (15.5)
145-154	149.5 (16.5)
155-164	159.5 (17.5)
165-174	169.5 (18.5)
175-184	179.5 (19.5)
185-194	189.5 (20.5)
195-204	199.5 (21.5)
205-214	209.5 (22.5)
215-224	219.5 (23.5)
225-234	229.5 (24.5)
235-244	239.5 (25.5)
245-254	249.5 (26.5)
255-264	259.5 (27.5)
265-274	269.5 (28.5)
275-284	279.5 (29.5)
285-294	289.5 (30.5)
295-304	299.5 (31.5)
305-314	309.5 (32.5)
315-324	319.5 (33.5)
325-334	329.5 (34.5)
335-344	339.5 (35.5)
345-354	349.5 (36.5)
355-364	359.5 (37.5)
365-374	369.5 (38.5)
375-384	379.5 (39.5)
385-394	389.5 (40.5)
395-404	399.5 (41.5)
405-414	409.5 (42.5)
415-424	419.5 (43.5)
425-434	429.5 (44.5)
435-444	439.5 (45.5)
445-454	449.5 (46.5)
455-464	459.5 (47.5)
465-474	469.5 (48.5)
475-484	479.5 (49.5)
485-494	489.5 (50.5)
495-504	499.5 (51.5)
505-514	509.5 (52.5)
515-524	519.5 (53.5)
525-534	529.5 (54.5)
535-544	539.5 (55.5)
545-554	549.5 (56.5)
555-564	559.5 (57.5)
565-574	569.5 (58.5)
575-584	579.5 (59.5)
585-594	589.5 (60.5)
595-604	599.5 (61.5)
605-614	609.5 (62.5)
615-624	619.5 (63.5)
625-634	629.5 (64.5)
635-644	639.5 (65.5)
645-654	649.5 (66.5)
655-664	659.5 (67.5)
665-674	669.5 (68.5)
675-684	679.5 (69.5)
685-694	689.5 (70.5)
695-704	699.5 (71.5)
705-714	709.5 (72.5)
715-724	719.5 (73.5)
725-734	729.5 (74.5)
735-744	739.5 (75.5)
745-754	749.5 (76.5)
755-764	759.5 (77.5)
765-774	769.5 (78.5)
775-784	779.5 (79.5)
785-794	789.5 (80.5)
795-804	799.5 (81.5)
805-814	809.5 (82.5)
815-824	819.5 (83.5)
825-834	829.5 (84.5)
835-844	839.5 (85.5)
845-854	849.5 (86.5)
855-864	859.5 (87.5)
865-874	869.5 (88.5)
875-884	879.5 (89.5)
885-894	889.5 (90.5)
895-904	899.5 (91.5)
905-914	909.5 (92.5)
915-924	919.5 (93.5)
925-934	929.5 (94.5)
935-944	939.5 (95.5)
945-954	949.5 (96.5)
955-964	959.5 (97.5)
965-974	969.5 (98.5)
975-984	979.5 (99.5)
985-994	989.5 (100.5)
995-1004	999.5 (101.5)
1005-1014	1009.5 (102.5)
1015-1024	1019.5 (103.5)
1025-1034	1029.5 (104.5)
1035-1044	1039.5 (105.5)
1045-1054	1049.5 (106.5)
1055-1064	1059.5 (107.5)
1065-1074	1069.5 (108.5)
1075-1084	1079.5 (109.5)
1085-1094	1089.5 (110.5)
1095-1104	1099.5 (111.5)
1105-1114	1109.5 (112.5)
1115-1124	1119.5 (113.5)
1125-1134	

The construction of the MOMP insert into the pET expression vector (Novagen, Inc.) yields, on transformation of permissive *E. coli*, an amino terminal thioredoxin fusion domain, a polyhistidine for Ni⁺-affinity chromatography, a solubility sequence of approximately 5 kD, and an endopeptidase cleavage site which yields a full length MOMP with a modified amino terminal (as illustrated in Figure 2) containing an alanine insert between the amino terminal methionine and the adjacent lysine. Either the full length expressed recombinant fusion protein or the modified MOMP following endopeptidase cleavage can be used as the antigen for a *Chlamydia* species ELISA. Other expression systems in *E. coli* or *Baculovirus* can be used for synthesis of the MOMP protein as the antigen in ELISA. The process is performed by non-covalent attachment of 50 ng recombinant MOMP in each well (rows 1-11) of a 96 well microtiter plate (Immulon 4) in carbonate buffer at pH 9.5 with an overnight incubation at 4°C. The plate is washed with PBS, 0.15% Tween20 x 3 and is then blocked with PBS, 1% BSA, 0.15% Tween, 20 at 300 ml per well for 1 hour at RT and then washed x 3 with PBS, 0.15% Tween20. Serum is serially diluted in PBS in triplicate in a separate plate and 50 µl of each well transferred to corresponding wells of a MOMP ligand plate, and the following sequence is followed: incubate at 37°C for 1 hour using a parafilm or other suitable cover to prevent non-uniform evaporation. Wash with PBS, 1% FCS, 0.05% NaN₃ x 5. Incubate each well with a predetermined dilution of biotin conjugated anti-human monoclonal IgG or monoclonal IgM. Incubate at 37°C for 1 hour with cover. Wash (x 3) with PBS, 1% FCS, 0.05% NaN₃. Follow with 50 µl strepavidin-alkaline phosphatase .

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conjugate (1:200 in PBS, 1% BSA, 0.15% Tween20) for 1 hour at 37°C with cover. Wash x 3 with PBS, 1% CS (calf serum), 0.05% NaN₃. Color is developed with p-nitrophenyl phosphate in glycine buffer at pH 9.6. The color yield is measured on a microtiter colorimeter using a 405 nm filter. The end point titer is the highest dilution of serum or secretion yielding a color yield >3 SD over background (n=8). Analysis is simplified by computer-generated end point antibody titer or other antibody level measure identification and/or quantity of specific antibody (IgG, IgM, or total Ig) in the test sample using appropriate controls. Other strepavidin or avidin enzyme conjugates can be substituted such as strepavidin peroxidase or strepavidin-galactosidase with an approximate substitute yielding a detectable color for quantitation.

b. Peptide-Based ELISA

The recombinant MOMP-based ELISA described above provides a sensitive method for the quantitation of immunoglobulins against the *Chlamydia* genus in serum, plasma, CSF, and other body fluids. In order to provide ELISA assays that are species- and potentially strain-specific for the various *Chlamydia*, two regions in the MOMP have been identified which show minimal amino acid sequence homologies and which are predicted by computer analysis (Intelligenetics) to be excellent antigenic domains by virtue of hydrophilicity and mobility on the solvent-accessible surface of MOMP. Figure 3 illustrates the constant and variable domain (VD) of the various chlamydial species. The identified species-specific antigenic domains are located in VD1 and VD2. Figure 4 illustrates the peptide amino acid sequences employed for the construction of peptide based ELISAs with species specificity for VD1. Figure 5 illustrates the peptides for VD2 which are used

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similarly to the VD1 sequences. ELISA methodology parallels that described above for the recombinant MOMP-based ELISA. In addition, a highly antigenic domain (Figure 6) common to all *Chlamydia* has been identified and
5 was developed as an alternative genus-specific ELISA for the *Chlamydia*. Immunization of rabbits has verified the antigenicity of each peptide to each peptide (Table 7). Monoclonal antibodies have further verified the specificities and antigenicity of each peptide (Table 7) as
10 predicted by computer analysis of the nucleotide-generated amino acid sequence of each species-specific MOMP.

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Table 7
Antigenic Responses To Peptides From 4 Species Of Chlamydiae Identified
By Hydrophilicity And Peptide Movement As Highly Antigenic

Chlamydiae Species	peptide ^b	Titer ^a	
		Pre	Post
<i>C. pneumoniae</i>	90-105	100	>3200
<i>C. trachomatis</i> L2	91-106	800	>3200
<i>C. psittaci</i>	92-106	400	>3200
<i>C. trachomatis</i> (mouse)	89-105	0	>3200

Chlamydiae Species	Peptide ^b	Titer ^a	
		Pre	Post
<i>C. pneumoniae</i>	158-171	25	>3200
<i>C. trachomatis</i> L2	159-175	200	>3200
<i>C. psittaci</i>	160-172	100	>3200
<i>C. trachomatis</i> (mouse)	158-171	800	>3200

Chlamydiae Species	Peptide ^b	Titer ^a	
		Pre	Post
<i>C. pneumoniae</i>	342-354	200	>3200
<i>C. trachomatis</i> L2	342-354	100	>3200
<i>C. psittaci</i>	ND ^c		
<i>C. trachomatis</i> (mouse)	ND ^c		

a Reciprocal titer

b Immunogenic peptide and ELISA antigen of specific amino acid sequence against the indicated pre-immunization and post-immunization rabbit serum

c ND, not done

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Table 8 illustrates reciprocal titers of a polyclonal and monoclonal antibody against *C. trachomatis* cross-reactive against *C. pneumoniae* peptide encompassing amino acids 342-354 and a recombinant full length MOMP from *C. pneumoniae*.

Table 8

Reciprocal titers of a polyclonal and a monoclonal antibody against *C. trachomatis* cross-reactive against *C. pneumoniae* peptide encompassing amino acids 342-354 and a recombinant full length MOMP from *C. pneumoniae*

Antigen	Titer ^a	
	Polyclonal Ab ^b	Monoclonal Ab ^c
CPN Momp ^d	400	0
CPN 90-105 ^e	50	0
CPN 158-171 ^f	50	0
CPN 342-354 ^g	>3200	1600

a Reciprocal titer

b Polyclonal goat Ab from Chemicon Inc. against MOMP of *C. trachomatis*

c Monoclonal Ab (ICN, Inc.) against MOMP of *C. trachomatis*

d *C. pneumoniae* recombinant MOMP

e Amino acid peptide 90-105 of *C. pneumoniae*

f Amino acid peptide 158-171 of *C. pneumoniae*

g Amino acid peptide 342-354 of *C. pneumoniae*

CPN 342-354

EXAMPLE 3

DETECTION ASSAY METHODS (DIAGNOSTIC)

a. Immunoglobulin (Ig) assay

C. pneumoniae EBs were grown in primary human
5 umbilical vein endothelial cells (HuEVEC; early passage),
HeLa 199, or a suitable alternative in the presence of 1
 $\mu\text{g/ml}$ cycloheximide at 35°C under 5% CO_2 . Permissive cells
were lysed at 3 days, thereby liberating EBs. The latter
were harvested from infection flasks, sonicated, and
10 cellular debris were removed after sonication by a low
speed centrifugation ($\sim 600 \times g$) for 5 minutes. EBs were
pelleted by high speed centrifugation ($30,000 \times g$) for 30
minutes at 4°C . The EB pellet was washed with PBS x1 and
was reconstituted in 2 ml PBS per four 25-cm^2 culture flask
15 and sonicated at maximum power for 20 seconds and a 0.5
cycle time using a Braun-Sonic U sonicator. EB protein
concentration was determined by the Bradford method and the
sonicated infectious EB suspension was rendered
non-infectious by the addition of 37% formaldehyde to a
20 final 10% formaldehyde concentration with constant
agitation during addition. Formalin-treated EBs were added
to 96-well plates at $50 \mu\text{l}$ per well containing 50 ng EB
(total of 5 $\mu\text{g/plate}$) and air dried. The plate was washed
with PBS-0.15% Tween20 x3 and was then blocked with PBS-1%
25 BSA-0.15% Tween20 at $300 \mu\text{l}$ per well for 1 hour at room
temperature and then washed x3 with PBS-0.15% Tween20.
Serum was serially diluted in PBS in duplicate in a
separate plate and $50 \mu\text{l}$ of each well transferred to corre-
sponding wells of a MOMP ligand plate and the following
30 sequence was followed: incubate at 37°C for 1 hour using a
parafilm cover; wash with PBS-1% FCS-0.05% NaN_3 x5;
incubate each well with a predetermined dilution of
biotin-conjugated, antihuman monoclonal IgG or monoclonal

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IgM; incubate at 37°C for 1 hour with cover; wash (x3) with PBS, 1% FCS, 0.05% NaN₃; follow with 50 µl strepavidin-alkaline phosphatase conjugate (1:200 in PBS-1% BSA-0.15% Tween20) for 1 hour at 37°C with cover; and wash
5 x3 with PBS, 1% CS, 0.05% NaN₃. Color was developed with p-nitrophenyl phosphate in glycine buffer at pH 9.6. The color yield was measured on a Flow microtiter colorimeter using a 405 nm filter. End point titer was the highest dilution of serum or secretion yielding a color yield > 3
10 SD over background (n = 8).

b. Western blot

Western blots were prepared by SDS-PAGE of *C. pneumoniae* EBs (non-formalin fixed) harvested from infected HuEVEC or HeLa cell lysates, electrophoresed under standard
15 SDS-PAGE conditions, and transferred to nitrocellulose achieved with an active diffusion transfer. Albumin-blocked strips were prepared from nitrocellulose sheets and incubated 1 hour with 1.2 ml of a 1:40 dilution of test serum. Detection was achieved with an alkaline
20 phosphatase-conjugated, mouse anti-human antibody, and developed with 5-bromo-4-chloro-3'-indolyphosphate p-toluidine/nitro-blue tetrazolium chloride (BCIP/NBT, Pierce Chemical Company). Polyclonal animal anti-human antibodies can alternatively be used.

25 c. Antigen Capture Assay for *Chlamydial* MOMP

The peptides described in Figures 3-5 were conjugated via disulfide bonding to keyhole limpet hemocyanin (KLH) by standard methods (Bernatowicz *et al.*, *Anal. Biochem.* 155(1):95-102 (1986)). The peptide conjugates in alum were
30 used to generate polyclonal and/or monoclonal antibodies to the species-specific domains of MOMP which is used as a capture antibody in 96 well microtiter plates. Final

configuration can follow a number of alternative routes to yield quantitation of MOMP in body fluids. The favored configuration utilizes biotin labeled recombinant MOMP in a competition assay with strepavidin/alkaline phosphatase generated color development based on the quantity of biotinylated recombinant MOMP displaced by unlabeled MOMP in body fluids.

EXAMPLE 4

IN VITRO ANTIMICROBIAL SUSCEPTIBILITY TESTING FOR C.

10 PNEUMONIAE

Tissue culture cells containing cryptic phase C. pneumoniae (H-292, HeLa, HEL, HuEVEC, McCoy, etc.) are plated at subconfluency in a 96-well microtiter plate (flasks or plates or other configurations can be alternatively used) and cultured in the presence of various antibiotics (singly and in combination) with the medium changed daily. Analysis of chlamydia activity is carried out by assessing loss of solution PCR signal, or relative activity can be quantified by dilution titer of the starting material using the absence of PCR signal as the endpoint titer (i.e., last dilution to yield specific PCR signal).

Two week exposure of single agents including the fluoroquinolone, ofloxacin, and the macrolide, clarithromycin, at 1 µg/ml failed to clear HeLa cells in culture of a detectable PCR signal for the MOMP gene of Chlamydia pneumoniae. In contrast, triple agents consisting of isoniazid (INH), metronidazole, and penicillamine (1 µg/ml each) resulted in no detectable PCR signal (Table 9). None of these agents, effective in the triple combination, is currently recognized as an anti-chlamydial agent.

Table 9. Susceptibility to Antibiotics for Cryptic *Chlamydia pneumoniae* Cultured in HeLa Cells^a

Antibiotic	Conc ($\mu\text{g/ml}$)	PCR ^b
Ofloxacin	1	positive
Clarithromycin	1	positive
INH	1	positive
Metronidazole	1	positive
Penicillamine	1/1	positive
INH + Metronidazole + Penicillamine	1/1/4 0	negative positive
Control		

- a Cultured in the presence of the indicated antibiotic(s), but with no cycloheximide. Media changes at 48-72 hours.
- b Analysis following 2 week exposure to antimicrobial agents.

Table 10
Susceptibility to Antibiotics for Cryptic
Chlamydia pneumoniae Cultured in HeLa Cells^a by PCR

Antibiotic	Conc (μ g/ml)	PCR 2 week	PCR 4 week
Minocycline	1	pos	pos
Doxycycline	1	pos	pos
Isoniazide	1	pos	pos
TMP/SMZ ^b	100	pos	pos
Minocycline + Metronidazole + penicillamine	1/1/4	pos	pos
Doxycyclin + Metronidazole + penicillamine	1/1/4	pos	neg
Isoniazid + Metronidazole + penicillamine	1/1/4	neg	neg
TMP/SMZ + Metronidazole + penicillamine	100/1/4	pos	neg
Metronidazole	0.25	pos	pos
Clarithromycin	0.25	pos	pos
Rifampin	0.25	pos	pos
Ofloxacin	0.25	pos	pos
Minocycline	0.25	pos	pos
Doxycycline	0.25	pos	pos
TMP/SMZ + Metronidazole	25/0.25	pos	pos
Ofloxacin + Metronidazole	0.25/0.25	pos	pos
Rifampin + Metronidazole + penicillamine	0.25/0.25/4	pos	pos
Rifampin + Metronidazole + Ofloxacin	0.25/0.25/0.25	pos	pos
Clarithromycin + Metronidazole + penicillamine	0.25/0.25/1	pos	neg
Doxycycline + Metronidazole + penicillamine	0.25/0.25/1	pos	pos
Minocycline + Metronidazole + penicillamine	0.25/0.25/1	pos	neg
Isoniazid + Metronidazole + penicillamine	0.25/0.25/1	neg	neg
TMP/SMZ	25	pos	pos
Rifampin + Metronidazole	0.25/0.25	pos	pos
None	0	pos	pos

^a Cultured in the presence of the indicated antibiotics, but with no cycloheximide. Media changes at 48-72 hours. pos = positive; neg = negative

^b TMP/SMZ = trimethoprim/sulfamethoxazole

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Table 11
Serological and PCR Responses to Combination Antibiotic Therapy

Patient	Diagnosis ^a	Titer		Months of Combination Antibiotic Therapy	PCR	Status
		IgM	IgG			
PH	FM	800 3200 800	800 1600 200	6 months	+ + wk+	Asymptomatic
BL	MS	2000 400	500 3200	9 months	+ wk+	Dramatic Improvement
MM	CFS/AND	3200 400	800 1600	1 month	+ +	Improvement; Relapse (non-compliant)
PM	CFS	2000 400	25 800	6 months	+ wk+	Asymptomatic
AM	IBD	800 3200	0 400	6 months	wk+ +	90% Improvement
FO	MS	800 800 400 400	3200 800 800 800	10 months	st+ + wk+ +	Improvement in speeds and bowl continence

VDB50 02pna

Table 11 (Continued)

Patient	Diagnosis ^a	Titer IgM IgG		Months of Combination Antibiotic Therapy	PCR	Status
WM	CF	25	25	Pre-illness serum <--Antibiotics start	wk+	Asymptomatic
		1000	25		st+	
		50	800		+	
		50	1600		wk+	
		50	400		-	
HM	CF	2000	100	6 months	+	Asymptomatic
		3200	3200		+	
		200	800		wk+	
CN	CFS	3200	800	8 months	+	75% Improvement
		800	800		wk+	
AN	MS/CFS	400	400		wk+	Strength ↑ Fatigue ↓
		200	3200		st+	
JS	CFS (severe)	2000	2000	5 months	st+	Asymptomatic
		2000	2000		+	
		200	800		-	
AG	IBD	3200	400	9 months	+	Improvement in joint Sx
		800	400		+	
		800	800		+	
		800	400		-	
AT	CF	3200	3200	9 months	+	Asymptomatic
		1600	1600		+	
		1600	1600		+	
		800	800		+	
		400	400		+	

Table 11 (Continued)

Patient	Diagnosis ^a	Titer		Months of Combination Antibiotic Therapy	PCR	Status
		IgM	IgG			
IH	RA	3200	1600	6 months	wk+	Improvement
		800	400		wk+	
		200	50		+	
HS	MS	2000	400	5 months	+	Improvement
		3200	800		+	
		50	200		-	
ST	CFS/FM	>1000	100	7 months	wk+	Asymptomatic
		1000	100		wk+	
		400	100		+	
		800	3200		+	
		100	100		+	
RV	CF	1000	100	10 months	+	Asymptomatic
		400	1600		+	
		400	400		-	

CF= Chronic Fatigue < 6 months

CFS=Chronic Fatigue Syndrome

FM=Fibromyalgia

IBD=Inflammatory Bowel Disease

MS=Multiple Sclerosis

AND=Autonomic nervous dysfunction (neural-mediated hypotension)

RA=Rheumatoid Arthritis

IgM >> IgG → immune tolerance to the antigen

IgG >> IgM → successful immune control of the antigen

Although the foregoing description is directed toward *Chlamydia*, it is merely for exemplary purposes and is not intended to limit the invention thereto. The invention therefore is relevant for other to obligate intracellular pathogens. For example, pathogens that must be in an intracellular location in order to replicate, include but are not limited to prions, viruses, *Chlamydiae* spp., *Mycoplasma* spp., *Ehrlichia* spp., *Rickettsia* spp., *Bartonella* spp., *Borrelia* spp., *Toxoplasma gondii*, *Leishmania* spp. and Trypanosomes (e.g., Malaria). Additionally, included are pathogens that are able to survive in an intracellular location and can find a physiologic advantage to do so, for example, *Legionella* spp., *Salmonella* spp., *Listeria* spp., *Histoplasma* spp., *Yersinia* spp. and *Mycobacteria* spp. Intracellular pathogens that are able to utilize selective intracellular locations to enhance survivability and/or pathogenics, are embraced in this invention and include but are not limited to *Neisseria* spp., *Staphylococcus* spp., *Hemophilus* spp., *Escherichia coli*, *Candida* spp. and *Torulopsis* spp.

EQUIVALENTS

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the claims.

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CLAIMS

We claim:

1. A combination of antichlamydial agents comprising at least two agents, each of which is effective against a different phase of chlamydial life cycle.
2. The combination according to Claim 1 wherein the agents are selected from the group consisting of:
 - a) agents effective against cryptic phase of chlamydial life cycle;
 - b) agents effective against elementary body phase of chlamydial life cycle; and
 - c) agents effective against replicating phase of chlamydial life cycle.
3. The combination according to Claim 1 wherein the agents are assembled as an admixture.
4. The combination according to Claim 1 wherein the agents are copackaged individually.
5. The combination according to Claim 1 wherein the agents are instructionally assembled.
6. The composition according to Claim 1 further comprising antiinflammatory agent, immunosuppressive agent, Vitamin C or combinations thereof.
7. A combination of antichlamydial agents for use in managing chlamydial infection or prophylaxis thereof; wherein the combination comprises at least two agents, each of which is effective against a different phase of chlamydial life cycle.

14. The pharmaceutical composition according to Claim 13 further comprising antiinflammatory agent, immunosuppressive agent, Vitamin C or combinations thereof.

5 15. A method for eliciting a protective immune response to *Chlamydia* infection in an animal or human by administering to an infected animal or human at least two agents, each of which is effective against a different phase of chlamydial life cycle until the human or animal no longer tests positive for *Chlamydia*.

16. A diagnostic kit or pack comprising materials adequate to perform one or more of the following:

15 a) antibody assays against recombinant MOMP;

b) antibody assays against specific antigenic peptides described in Figures 3, 4 and 5;

c) antigen capture assays directed against MOMP or the peptides described in Figures 3, 4 and 5;

20 d) DNA amplification assays for chlamydial genes; and

e) Western blot used as a confirmatory.

17. The kit or pack of Claim 16, wherein the antibody assays are ELISAs.

25 18. A pharmaceutical pack of therapeutic agents for management of *Chlamydia* infection, comprising at least two agents, each of which is effective against a different phase of chlamydial life cycle.

19. The pharmaceutical pack according to Claim 18 wherein the agents are selected from the group consisting of:

27. The method according to Claim 26 wherein the agents are selected from the group consisting of:
- a) agents effective against cryptic phase of chlamydial life cycle;
 - 5 b) agents effective against elementary body phase of chlamydial life cycle; and
 - c) agents effective against replicating phase of chlamydial life cycle.
28. The method according to Claim 26 wherein the agent effective against the elementary body phase is a disulfide reducing agent.
29. The method according to Claim 29, wherein the agent effective against the cryptic phase is a nitroaromatic compound.
- 15 30. The method according to Claim 26, wherein the nitroaromatic compound is selected from the group consisting of nitroimidazoles, nitrofurans, analogs, derivative and combinations thereof.
31. A method for managing chlamydial infection in an individual in need thereof, comprising administering at least two agents, each of which is effective against a different phase of chlamydial life cycle.
- 20 32. The method according to Claim 31 wherein the agents are selected from the group consisting of:
- 25 a) agents effective against cryptic phase of chlamydial life cycle;
 - b) agents effective against elementary body phase of chlamydial life cycle; and

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c) agents effective against replicating phase of chlamydial life cycle.

33. A method for diagnosing disease associated with *Chlamydia* infection comprising diagnosing the *Chlamydia* infection in an individual, wherein the disease is an autoimmune disease, an inflammatory diseases or a disease that occurs in immunocompromised individuals.
34. A method for treating disease associated with *Chlamydia* infection comprising treating the *Chlamydia* infection in an individual in need thereof, comprising administering a combination of antichlamydial agents that are effective against at least two phases of the chlamydial life cycle. wherein the disease is an autoimmune disease, an inflammatory diseases and disease that occurs in immunocompromised individuals.
35. The method according to Claim 34 wherein the agents are selected from at least two different agents from at least two of the following groups:
- a) agents effective against cryptic phase of chlamydial life cycle;
 - b) agents effective against elementary body phase of chlamydial life cycle; and
 - c) agents effective against replicating phase of chlamydial life cycle.
36. The method according to Claim 35 wherein the infection is a *Chlamydia pneumoniae* infection.

37. An assay for identifying an agent which is capable of inhibiting chlamydial infection, comprising the steps of:

- a) preparing tissue culture cells infected with *Chlamydia* in the absence of cycloheximide;
- b) allowing the *Chlamydia* to replicate;
- c) adding a test agent;
- d) isolating chlamydial nucleic acid from the cells;
- e) amplifying the chlamydial nucleic acid by a nucleic acid amplification technique; and
- f) evaluating the presence or absence of amplified chlamydial nucleic acid;

wherein the absence of amplified chlamydial nucleic acids is indicative that the agent is capable of inhibiting chlamydial infection.

38. The assay of Claim 37 wherein the amplification technique is PCR.

39. A method of identifying cells containing cryptic form of *Chlamydia* comprising the steps of:

- a) treating cultured cells, thought to be infected with chlamydia, with a disulfide reducing agent;
- b) subjecting cultured cells to protease digestion;
- c) exposing cells to appropriate polymerase, dNTPs and primers for DNA amplification of a chlamydial protein;
- d) exposing the cells to a reporter molecule enzyme;
- e) exposing the cells to an appropriate substrate for the reporter enzyme; and
- f) determining the presence of cryptic form of *Chlamydia* by visualizing the amplified DNA encoding a chlamydial protein.

40. A method according to Claim 39, wherein the *Chlamydia* is *Chlamydia pneumoniae*.

41. A method according to Claim 40, wherein the primers of step c) are CHLMOMPDB2 and CHLMOMPCB2.

5 42. A method of identifying cells containing cryptic form of *Chlamydia* comprising the steps of:

- a) treating cultured cells, thought to be infected with chlamydia, with guanidine thiocyanate;
- b) exposing cells to appropriate polymerase, dNTPs and primers for DNA amplification of a chlamydial protein;
- c) exposing the cells to a reporter molecule enzyme;
- d) exposing the cells to an appropriate substrate for the reporter enzyme; and
- 15 e) determining the presence of cryptic form of *Chlamydia* by visualizing the amplified DNA encoding a chlamydial protein.

43. An assay for identifying an agent which is effective against cryptic form of *Chlamydia* comprising the steps of:

- a) treating cultured cells grown in the absence of cycloheximide, thought to be infected with chlamydia, with a disulfide reducing agent;
- b) allowing the chlamydia to replicate;
- 25 c) adding a test agent;
- d) subjecting cultured cells to protease digestion;
- e) exposing cells to appropriate polymerase, dNTPs and primers for DNA amplification of a chlamydial protein;
- 30 f) exposing the cells to a reporter molecule enzyme;

amplification technique to detect chlamydial DNA in the sample.

48. A method of detecting chlamydial elementary bodies in a sample comprising performing on the sample an
5 antigen capture assay directed against MOMP or the peptides described in Figures 3, 4 and 5.

49. The method of Claim 48, wherein the sample is contacted with a disulfide reducing agent before the assay is performed.

10 50. A peptide of Figure 3, 4 and 5.

51. Antichlamydia agent identified by the assay of Claim 37.

52. Antichlamydia agent identified by the assay of Claim 43.

15 53. The method of Claim 46 wherein the agent effective against the cryptic phase is a nitroaromatic compound.

54. The method of Claim 46 wherein the agent effective against the elementary body phase is a disulfide
20 reducing agent.

55. A method of determining the status of a patient or monitoring and/or modifying the course of therapy for chlamydia infection comprising the results of one or more assays made contemporaneously or sequentially,
25 wherein the assays are selected from the group consisting of:

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- 5 a) antibody assays against recombinant MOMP;
b) antibody assays against specific antigenic
 peptides described in Figures 3, 4 and 5;
c) antigen capture assays directed against MOMP or
 the peptides described in Figures 3, 4 and 5;
d) DNA amplification assays for chlamydial genes;
 and
e) Western blot used as a confirmatory.
- 10 56. The method of Claim 55, wherein the antibody assays
 are ELISAs.
57. The method of Claim 55 wherein DNA amplification
 assay is PCR.
58. The method of Claim 55 wherein DNA amplification
 assay amplifies the MOMP gene.
- 15 59. The method of Claim 55 wherein more than one assay is
 used.
60. The method of Claim 55 wherein specific antibody
 measures are obtained against IgA, IgE, IgM and/or
 IgG.
- 20 61. An assay for identifying an agent which is capable of
 inhibiting chlamydial infection in a patient or an
 animal, comprising the steps of:
- 25 a) obtaining a biological material sample from a
 patient from which the infectious organism is
 extracted and applied to target cells for
 culturing;
 b) allowing the *Chlamydia* to replicate;
 c) adding one or more agents;

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- d) isolating chlamydial nucleic acid from the cells with the use of protease and reducing agents, or guanidine thiocyanate;
- e) amplifying the chlamydial nucleic acid by a nucleic acid amplification technique; and
- f) evaluating the presence or absence of amplified chlamydial nucleic acid;

wherein the absence of amplified chlamydial nucleic acids is indicative that the agent or agents is capable of inhibiting chlamydial infection.

62. A method according to Claim 61 wherein the *Chlamydia* is *Chlamydia pneumonia*.

63. A method according to Claim 61 wherein the primers of step d) are CHLMOMPDB2 and CHLMOMPCB2.

64. A method according to Claim 61 wherein at least two or more probes are used to reduce false positives.

65. A method of treating a chlamydial infection or disease associated therewith comprising treating the patient to reduce the acellular load of infectious EBs.

66. A method of treating a chlamydial infection or disease associated therewith comprising treating the patient with one or more antibiotics for not less than 45 days.

67. A method for determining the effectiveness of an antichlamydial agent, comprising the steps of:

a) obtaining serum or whole blood from an animal or human that has undergone therapy for eliminating

Leader

SEQ ID NO: 45 L2
46 B
47 D
48 E
49 L1
50 F
51 C
52 A
53 H
54 L3
55 MU
56 PN

Leader

SEQ ID NO: 45 L2
46 B
47 D
48 E
49 L1
50 F
51 C
52 A
53 H
54 L3
55 MU
56 PN

VD1

Leader

SEQ ID NO: 45 L2
46 B
47 D
48 E
49 L1
50 F
51 C
52 A
53 H
54 L3
55 MU
56 PN

VD1

VD 2

SEQ ID NO: 57 L2
58 B
59 D
60 E
61 L1
62 F
63 C
64 A
65 H
66 L3
67 MU
68 PN

VD 2

SEQ ID NO: 57 L2
58 B
59 D
60 E
61 L1
62 F
63 C
64 A
65 H
66 L3
67 MU
68 PN

VD 2

FIG. 1A

Figure 1 consists of 12 bar charts, labeled (a) through (l), each representing a different demographic or attitudinal variable. Each chart has two bars: a solid black bar representing the percentage of respondents in the sample and a white bar with a black outline representing the percentage of the total population. The y-axis for all charts ranges from 0 to 100 percent.

- (a) Age:** The respondent bar is slightly above the population bar for all age groups (18-24, 25-34, 35-44, 45-54, 55-64, 65+).
- (b) Sex:** The respondent bar is slightly above the population bar for both males and females.
- (c) Education:** The respondent bar is slightly above the population bar for all education levels (High school or less, Some college, Bachelor's degree, Master's degree, Doctorate).
- (d) Income:** The respondent bar is slightly above the population bar for all income levels (Less than \$10,000, \$10,000-\$19,999, \$20,000-\$29,999, \$30,000-\$39,999, \$40,000-\$49,999, \$50,000-\$59,999, \$60,000-\$69,999, \$70,000-\$79,999, \$80,000-\$89,999, \$90,000-\$99,999, \$100,000+).
- (e) Employment:** The respondent bar is slightly above the population bar for all employment status categories (Unemployed, Part-time, Full-time, Retired, Other).
- (f) Home ownership:** The respondent bar is slightly above the population bar for both homeowners and renters.
- (g) Political affiliation:** The respondent bar is slightly above the population bar for all political affiliations (Democrat, Republican, Independent, Other).
- (h) Party affiliation:** The respondent bar is slightly above the population bar for all party affiliations (Democrat, Republican, Independent, Other).
- (i) Party identification:** The respondent bar is slightly above the population bar for all party identifications (Democrat, Republican, Independent, Other).
- (j) Party loyalty:** The respondent bar is slightly above the population bar for all party loyalties (Democrat, Republican, Independent, Other).
- (k) Party support:** The respondent bar is slightly above the population bar for all party supports (Democrat, Republican, Independent, Other).
- (l) Party preference:** The respondent bar is slightly above the population bar for all party preferences (Democrat, Republican, Independent, Other).

VD 3

[illegible]

VD 4

[illegible]

FIG. 1B.

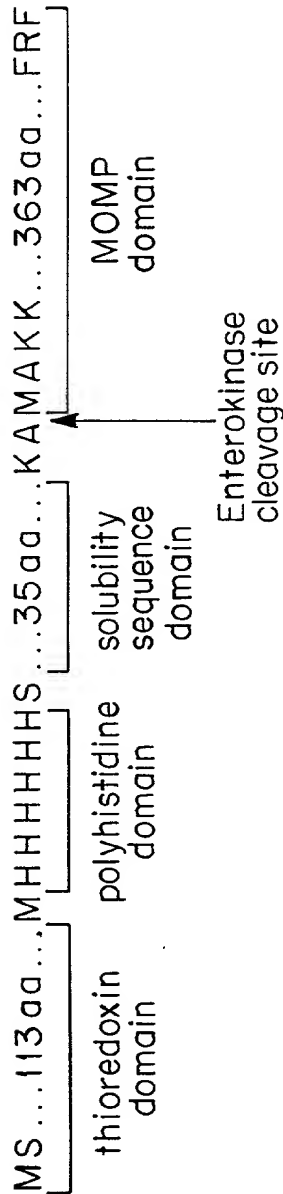


FIG. 2

	SEQ ID NO.																			
CPN90-105 <u>C. pneumoniae</u>	C	T	G	S	A	A	-	A	N	Y	T	T	A	V	D	-	R	P	N	93
CTP89-105 <u>C. trachomatis</u> (mouse)	C	T	G	D	A	D	L	T	T	A	P	T	P	A	S	-	R	E	N	94
CTL91-106 <u>C. trachomatis</u> (L2)	C	T	T	A	T	G	N	A	A	A	P	S	T	C	T	A	R	E	N	95
CPS92-106 <u>C. psittaci</u>	C	A	S	G	T	A	-	S	N	T	T	V	A	A	D	-	R	S	N	96

FIG. 3

	SEQ IDNO.																		
CPN158-171 <u>C. pneumoniae</u>	C	F	G	V	K	G	T	T	V	N	A	N	E	-	-	L	P	97	
CTP158-171 <u>C. trachomatis</u> (mouse)	C	F	G	R	D	E	T	A	V	A	A	D	D	-	-	I	P	98	
CTL159-175 <u>C. trachomatis</u> (L2)	C	F	G	D	N	E	N	H	A	T	V	S	D	S	K	L	V	P	99
CPS160-172 <u>C. psittaci</u>	C	I	G	L	A	G	T	D	F	-	A	N	Q	-	-	-	R	P	100

FIG. 4

													SEQ ID NO.	
CPN342-354 <u>C. pneumoniae</u>	C	Q	I	N	K	F	K	S	R	K	A	C	G	I 01
CTP342-354 <u>C. trachomatis</u> (mouse)	C	Q	I	N	K	M	K	S	R	F	A	C	G	I 02
CTL342-354 <u>C. trachomatis</u> (L2)	C	Q	L	N	K	M	K	S	R	K	A	C	G	I 03
CPS342-354 <u>C. psittaci</u>	C	Q	I	N	K	F	K	S	R	F	A	C	G	I 04

FIG. 5

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled DIAGNOSIS AND MANAGEMENT OF INFECTION CAUSED BY CHLAMYDIA, the specification of which

- ☒ is attached hereto.
☐ was filed on _____ as Application Serial No. _____
and was amended on _____.
☐ was described and claimed in PCT International Application No. _____
filed on _____ and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56(a).

NON-PROVISIONAL PRIORITY RIGHTS: I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information I know to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Serial Number	Filing Date	Status
09/025,521	February 18, 1998	Pending

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Paul T. Clark, Reg. No. 30,162, Karen L. Elbing, Ph.D. Reg. No. 35,238, Kristina Bieker-Brady, Ph.D. Reg. No. 39,109, Susan M. Michaud, Ph.D. Reg. No. 42,885, Mary Rose Scozzafava, Ph.D., Reg. No.36,268, James D. DeCamp, Ph.D., Reg. No. 43,580, Sean J. Edman, Reg. No. 42,506, Timothy J. Douros, Reg. No. 41,716.

Address all telephone calls to: Karen L. Elbing, Ph.D. at 617/428-0200.

Address all correspondence to: Karen L. Elbing, Ph.D. at Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110.

COMBINED DECLARATION AND POWER OF ATTORNEY

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
William M. Mitchell	Nashville, Tennessee	251 Vaughns Gap Road Nashville, TN 37205	USA
Signature:			Date:

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
Charles W. Stratton	Nashville, Tennessee	207 Kensington Park Nashville, TN 37215	USA
Signature:			Date:

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGAAAAAAC TCTTGAAATC GGCATTATTG TTTGCCGCTA CGGGTTCCGC 50

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATGAAAAAAC TCTTGAAATC GGCATTATTG TTTGCCGCTA CGGGTTCCGC 50

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGAAAAAAC TCTTGAAATC GGCATTATTG TTTGCCGCTA CGGGTTCCGC 50

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGAAAAAAC TCTTGAAATC GGCATTATTA TTTGCCGCTA CGGGTTCCGC 50

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(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGAAAAAAC TCTTAAATC GGCATTATTA TTTGCCGCTG CGGGTTCCGC

50

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATGAAAAAAC TCTTGAAATC GGTATTAGTA TTTGCCGCTT TGAGTTCTGC

50

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGAAAAAAC TCTTGAAATC GGTATTAGTA TTTGCCGCTT TGAGTTCTGC

50

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATGAAAAAAC TCTTGAAATC GGTATTAGTA TTTGCCGCTT TGAGTTCTGC

50

(2) INFORMATION FOR SEQ ID NO:13:

003077-10260260

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGAAAAAAC TCTTGAAATC GGTATTAGTA TTTGCCGCTT TGAGTTCTGC

50

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATGAAAAAAC TCTTGAAATC GGTATTAGTA TTTGCCGCTT TGAGTTCTGC

50

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATGAAAAAAC TCTTGAAATC GGTATTAGTA TTTGCCGCTT TGAGTTCTGC

50

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ATGAAAAAAC TCTTGAAATC GGTATTAGTG TTTGCCGCTT TGAGTTCTGC

50

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(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CGCAGTTACA GTTGAGACTC GCTTGATCGA TGAGAGAGCA GCTCACGTAA

50

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GCTTGATCGA TGAGAGAGCA GGTACAGTAA ATGCACAATT CCGGTTCTAA

50

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GCTTGATCGA TGAGAGAGCA GCTCACGTAA ATGCACAATT CCGCTTCTAA

50

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CGCTTGATCG ATGAGAGACT GCTCACGTAA ATGCACAATT CCGCTTCTAA

50

CGCTTGATCG ATGAGAGACT GCTCACGTAA ATGCACAATT CCGCTTCTAA

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GCTTGATCGA TGAGAGAGCT GCTCACGTAA ATGCACAATT CCGCTTCTAA

50

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GCTTGATCGA TGAGAGAGCA GCTCACGTAA ATGCACAATT CCGCTTCTAA

50

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GCTTGATCGA TGAGAGAGCT GCTCACGTAA ATGCACAATT CCGCTTCTAA

50

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 49 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CTTGATCGAT GAGAGAGCTG CTCACGTAAA TGCACAATTC CGCTTCTAA

49

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(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 134 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Cys Thr Ala Arg Glu Asn Pro Ala Tyr Gly Arg His Met Gln Asp Ala
 1 5 10 15
 Glu Met Phe Thr Asn Cys Ala Tyr Met Ala Leu Ile Asn Trp Asp Arg
 20 25 30
 Phe Asp Val Phe Cys Thr Leu Gly Ala Thr Ser Gly Tyr Leu Lys Gly
 35 40 45
 Asn Ser Ala Ser Phe Asn Leu Val Gly Leu Phe Gly Asp Asn Glu Asn
 50 55 60
 Gln Lys Thr Val Lys Ala Glu Ser Val Pro Asn Met Ser Phe Asp Gln
 65 70 75 80
 Ser Val Val Glu Leu Tyr Thr Asp Thr Thr Phe Ala Trp Ser Val Gly
 85 90 95
 Ala Arg Ala Thr Lys Val Ser Asn Gly Thr Phe Val Pro Asn Met Ser
 100 105 110
 Leu Asp Gln Ser Val Val Glu Leu Tyr Thr Asp Thr Ala Phe Ala Trp
 115 120 125
 Ser Val Gly Ala Arg Ala
 130

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 99 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Leu Thr Ala Arg Glu Asn Pro Ala Tyr Gly Arg His Met Gln Asp Ala
 1 5 10 15
 Glu Met Phe Thr Asn Cys Ala Tyr Met Ala Leu Ile Asn Trp Asp Arg
 20 25 30
 Phe Asp Val Phe Cys Thr Leu Gly Ala Ser Ser Gly Tyr Leu Lys Gly
 35 40 45
 Asn Ser Ala Ser Phe Asn Leu Val Gly Leu Phe Gly Asp Asn Glu Asn
 50 55 60

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Gln Ser Thr Val Lys Thr Asn Ser Val Pro Asn Met Ser Leu Asp Gln
 65 70 75 80
 Ser Val Val Glu Leu Tyr Thr Asp Thr Ala Phe Ser Trp Ser Val Gly
 85 90 95
 Ala Arg Ala

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 99 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Cys Thr Ala Arg Glu Asn Pro Ala Tyr Gly Arg His Met Gln Asp Ala
 1 5 10 15
 Glu Met Phe Thr Asn Ala Ala Tyr Met Ala Leu Ile Asn Trp Asp Arg
 20 25 30
 Phe Asp Val Phe Cys Thr Leu Gly Ala Thr Ser Gly Tyr Leu Lys Gly
 35 40 45
 Asn Ser Ala Ser Phe Asn Leu Val Gly Leu Phe Gly Asp Asn Glu Asn
 50 55 60
 Gln Ser Thr Val Lys Lys Asp Ala Val Pro Asn Met Ser Phe Asp Gln
 65 70 75 80
 Ser Val Val Glu Leu Tyr Thr Asp Thr Thr Phe Ala Trp Ser Val Gly
 85 90 95
 Ala Arg Ala

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 99 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Leu Val Glu Arg Thr Asn Pro Ala Tyr Gly Lys His Met Gln Asp Ala
 1 5 10 15
 Glu Met Phe Thr Asn Cys Ala Tyr Thr Ala Leu Ile Asn Trp Asp Arg
 20 25 30
 Phe Asp Val Phe Cys Thr Leu Gly Ala Thr Ser Gly Tyr Leu Lys Gly
 35 40 45
 Asn Ser Ala Ser Phe Asn Leu Val Gly Leu Phe Gly Asp Gly Val Asn
 50 55 60

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[illegible]

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

[illegible]

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Asn	Val	Ala	Arg	Pro	Asn	Pro	Ala	Tyr	Gly	Lys	His	Met	Gln	Asp	Ala
1				5					10					15	
Glu	Met	Phe	Thr	Asn	Ala	Ala	Tyr	Met	Ala	Leu	Ile	Asn	Trp	Asp	Arg
			20					25					30		
Phe	Asp	Val	Phe	Cys	Thr	Leu	Gly	Ala	Thr	Thr	Gly	Tyr	Leu	Lys	Gly
		35					40					45			
Asn	Ser	Ala	Ser	Phe	Asn	Leu	Val	Gly	Leu	Phe	Gly	Thr	Lys	Thr	Gln
	50					55					60				

Ser Ser Gly Phe Asp Thr Ala Asn Ile Val Pro Asn Thr Ala Leu Asn
65 70 75 80
Gln Ala Val Val Glu Leu Tyr Thr Asp Thr Phe Ala Trp Ser Val
85 90 95
Gly Ala Arg Ala
100

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

[illegible]

(2) INFORMATION FOR SEO ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Asn	Val	Ala	Arg	Pro	Asn	Pro	Ala	Tyr	Gly	Lys	His	Met	Gln	Asp	Ala
1				5					10					15	
Glu	Met	Phe	Thr	Asn	Ala	Ala	Tyr	Met	Ala	Leu	Ile	Asn	Trp	Asp	Arg
			20					25					30		
Phe	Asp	Val	Phe	Cys	Thr	Leu	Gly	Ala	Thr	Thr	Gly	Tyr	Leu	Lys	Gly
		35					40					45			
Asn	Ser	Ala	Ser	Phe	Asn	Leu	Val	Gly	Leu	Phe	Gly	Thr	Lys	Thr	Gln
	50					55					60				

Ser Thr Asn Phe Asn Thr Ala Lys Leu Val Pro Asn Thr Ala Leu Asn
 65 70 75 80
 Gln Ala Val Val Glu Leu Tyr Thr Asp Thr Thr Phe Ala Trp Ser Val
 85 90 95
 Gly Ala Arg Ala
 100

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 96 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Ala Ser Arg Glu Asn Pro Ala Tyr Gly Lys His Met Gln Asp Ala Glu
 1 5 10 15
 Met Phe Thr Asn Ala Ala Tyr Met Ala Leu Ile Asn Trp Asp Arg Phe
 20 25 30
 Asp Val Phe Cys Thr Leu Gly Ala Thr Ser Gly Tyr Leu Lys Gly Asn
 35 40 45
 Ser Ala Ala Phe Asn Leu Val Gly Leu Phe Gly Arg Asp Glu Thr Ala
 50 55 60
 Val Ala Ala Asp Asp Ile Pro Asn Val Ser Leu Ser Gln Ala Val Val
 65 70 75 80
 Glu Leu Tyr Thr Asp Thr Ala Phe Ala Trp Ser Val Gly Ala Arg Ala
 85 90 95

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Tyr Thr Thr Ala Val Asp Arg Pro Asn Pro Ala Tyr Asn Lys His Leu
 1 5 10 15
 His Asp Ala Glu Trp Phe Thr Asn Ala Gly Ile Phe Ala Leu Ile Asn
 20 25 30
 Trp Asp Arg Phe Asp Val Phe Cys Thr Leu Gly Ala Ser Asn Gly Ile
 35 40 45
 Arg Lys Gly Asn Ser Thr Ala Phe Asn Leu Val Gly Leu Phe Gly Val
 50 55 60
 Lys Gly Thr Thr Val Asn Ala Asn Glu Leu Pro Asn Val Ser Leu Ser
 65 70 75 80

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Asn Gly Val Val Glu Leu Tyr Thr Asp Thr Ser Phe Ser Trp Ser Val
85 90 95
Gly Ala Arg Ala
100

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

[illegible]

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Ala	Leu	Trp	Glu	Cys	Gly	Cys	Ala	Thr	Leu	Gly	Ala	Ser	Phe	Gln	Tyr
1				5					10					15	
Ala	Gln	Ser	Lys	Pro	Lys	Val	Glu	Glu	Leu	Asn	Val	Leu	Cys	Asn	Ala
			20					25					30		
Ala	Glu	Phe	Thr	Ile	Asn	Lys	Pro	Lys	Gly	Tyr	Val	Gly	Lys	Glu	Leu
		35					40					45			
Pro	Leu	Asp	Leu	Thr	Ala	Gly	Thr	Asp	Ala	Ala	Thr	Gly	Thr	Lys	Asp
	50					55					60				
Ala	Ser	Ile	Asp	Tyr	His	Glu	Trp	Gln	Ala	Ser	Leu	Ala	Leu	Ser	Tyr
65					70					75					80

Arg Leu Asn Met Phe Thr Pro Tyr Ile Gly Val Lys Trp Ser Arg Ala
85 90 95
Ser Phe Asp Ala
100

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

[illegible]

(2) INFORMATION FOR SEO ID NO:72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Ala	Leu	Trp	Glu	Cys	Gly	Cys	Ala	Thr	Leu	Gly	Ala	Ser	Phe	Gln	Tyr
1				5					10					15	
Ala	Gln	Ser	Lys	Pro	Lys	Val	Glu	Glu	Leu	Asn	Val	Leu	Cys	Asn	Ala
			20					25					30		
Ala	Glu	Phe	Thr	Ile	Asn	Lys	Pro	Lys	Gly	Tyr	Val	Gly	Gln	Glu	Phe
		35					40					45			
Pro	Leu	Ala	Leu	Ile	Ala	Gly	Thr	Asp	Ala	Ala	Thr	Gly	Thr	Lys	Asp
	50					55					60				
Ala	Ser	Ile	Asp	Tyr	His	Glu	Trp	Gln	Ala	Ser	Leu	Ala	Leu	Ser	Tyr
65					70					75					80

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Arg Leu Asn Met Phe Thr Pro Tyr Ile Gly Val Lys Trp Ser Arg Ala
85 90 95
Ser Phe Asp Ala
100

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Ala Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Ala Ser Phe Gln Tyr
1 5 10 15
Ala Gln Ser Lys Pro Lys Val Glu Glu Leu Asn Val Leu Cys Asn Ala
20 25 30
Ala Glu Phe Thr Ile Asn Lys Pro Lys Gly Tyr Val Gly Lys Glu Phe
35 40 45
Pro Leu Asp Leu Thr Ala Gly Thr Asp Ala Ala Thr Gly Thr Lys Asp
50 55 60
Ala Ser Ile Asp Tyr His Glu Trp Gln Ala Ser Leu Ala Leu Ser Tyr
65 70 75 80
Arg Leu Asn Met Phe Thr Pro Tyr Ile Gly Val Lys Trp Ser Arg Ala
85 90 95
Ser Phe Asp Ala
100

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Ala Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Ala Ser Phe Gln Tyr
1 5 10 15
Ala Gln Ser Lys Pro Lys Ile Glu Glu Leu Asn Val Leu Cys Asn Ala
20 25 30
Ala Glu Phe Thr Ile Asn Lys Pro Lys Gly Tyr Val Gly Lys Glu Phe
35 40 45
Pro Leu Asp Leu Thr Ala Gly Thr Asp Ala Ala Thr Gly Thr Lys Asp
50 55 60
Ala Ser Ile Asp Tyr His Glu Trp Gln Ala Ser Leu Ser Leu Ser Tyr
65 70 75 80

(2) INFORMATION FOR SEQ ID NO:75:

(A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEO ID NO:75:

(2) INFORMATION FOR SEO ID NO:76:

(A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

Ala	Leu	Trp	Glu	Cys	Gly	Cys	Ala	Thr	Leu	Gly	Ala	Ser	Phe	Gln	Tyr
1				5					10					15	
Ala	Gln	Ser	Lys	Pro	Lys	Val	Glu	Glu	Leu	Asn	Val	Leu	Cys	Asn	Ala
			20					25					30		
Ser	Glu	Phe	Thr	Ile	Asn	Lys	Pro	Lys	Gly	Tyr	Val	Gly	Ala	Glu	Phe
		35					40					45			
Pro	Leu	Asp	Ile	Thr	Ala	Gly	Thr	Glu	Ala	Ala	Thr	Gly	Thr	Lys	Asp
	50					55					60				

Ala Ser Ile Asp Tyr His Glu Trp Gln Ala Ser Leu Ala Leu Ser Tyr
 65 70 75 80
 Arg Leu Asn Met Phe Thr Pro Tyr Ile Gly Val Lys Trp Ser Arg Val
 85 90 95
 Ser Phe Asp Ala
 100

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

Ala Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Ala Ser Phe Gln Tyr
 1 5 10 15
 Ala Gln Ser Lys Pro Lys Val Glu Glu Leu Asn Val Leu Cys Asn Ala
 20 25 30
 Ser Glu Phe Thr Ile Asn Lys Pro Lys Gly Tyr Val Gly Ala Glu Phe
 35 40 45
 Pro Leu Asp Ile Thr Ala Gly Thr Glu Ala Ala Thr Gly Thr Lys Asp
 50 55 60
 Ala Ser Ile Asp Tyr His Glu Trp Gln Ala Ser Leu Ala Leu Ser Tyr
 65 70 75 80
 Arg Leu Asn Met Phe Thr Pro Tyr Ile Gly Val Lys Trp Ser Arg Val
 85 90 95
 Ser Phe Asp Ala
 100

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Ala Leu Trp Glu Cys Gly Cys Ala Thr Leu Gly Ala Ser Phe Gln Tyr
 1 5 10 15
 Ala Gln Ser Lys Pro Lys Val Glu Glu Leu Asn Val Leu Cys Asp Ala
 20 25 30
 Ser Glu Phe Thr Ile Asn Lys Pro Lys Gly Tyr Val Gly Ala Glu Phe
 35 40 45
 Pro Leu Asp Ile Thr Ala Gly Thr Glu Ala Ala Thr Gly Thr Lys Asp
 50 55 60

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Ala Ser Ile Asp Tyr His Glu Trp Gln Ala Ser Leu Ala Leu Ser Tyr
65                               70                         75           80
Arg Leu Asn Met Phe Thr Pro Tyr Ile Gly Val Lys Trp Ser Arg Val
                        85                               90                 95
Ser Phe Asp Ala
                          100
```

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

[illegible]

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Gly	Leu	Trp	Glu	Cys	Gly	Cys	Ala	Thr	Leu	Gly	Glu	Ser	Phe	Gln	Tyr
1				5					10					15	
Ala	Gln	Ser	Lys	Pro	Lys	Val	Glu	Glu	Leu	Asn	Val	Ile	Cys	Asn	Val
			20					25					30		
Ser	Gln	Phe	Ser	Val	Asn	Lys	Pro	Lys	Gly	Tyr	Lys	Gly	Val	Ala	Phe
		35					40					45			
Pro	Leu	Pro	Thr	Asp	Ala	Gly	Val	Ala	Thr	Ala	Thr	Gly	Thr	Lys	Ser
	50					55					60				

Ala Thr Ile Asn Tyr His Glu Trp Gln Val Gly Ala Ser Leu Ser Tyr
65 70 75 80
Arg Leu Asn Ser Leu Val Pro Tyr Ile Gly Val Gln Trp Ser Arg Ala
 85 90 95
Thr Phe Asp Ala
 100

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Asp 1	Thr	Ile	Arg	Ile 5	Ala	Gln	Pro	Lys	Ser 10	Ala	Thr	Thr	Val	Phe 15	Asp
Val	Thr	Thr	Leu	Asn 20	Pro	Thr	Ile	Ala 25	Gly	Ala	Gly	Asp	Val 30	Lys	Ala
Ser	Ala	Glu	Gly	Gln	Leu	Gly	Asp 40	Thr	Met	Gln	Ile	Val 45	Ser	Leu	Gln
Leu	Asn 50	Lys	Met	Lys	Ser	Arg 55	Lys	Ser	Cys	Gly	Ile 60	Ala	Val	Gly	Thr
Thr 65	Ile	Val	Asp	Ala 70	Asp	Lys	Tyr	Ala	Val 75	Thr	Val	Glu	Thr	Arg	Leu 80
Ile	Asp	Glu	Arg	Ala 85	Ala	His	Val	Asn 90	Ala	Gln	Phe	Arg	Phe		

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 92 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

Asp	Thr	Ile	Arg	Ile	Ala	Gln	Pro	Lys	Ser	Ala	Glu	Thr	Ile	Phe	Asp
1				5					10					15	
Val	Thr	Thr	Leu	Asn	Pro	Thr	Ile	Ala	Gly	Ala	Gly	Asp	Val	Lys	Thr
			20					25					30		
Ser	Ala	Glu	Gly	Gln	Leu	Gly	Asp	Thr	Met	Gln	Ile	Val	Ser	Leu	Gln
		35					40					45			
Leu	Asn	Met	Lys	Ser	Arg	Lys	Cys	Gly	Ile	Ala	Val	Gly	Thr	Thr	Ile
	50					55					60				

Val Asp Ala Asp Lys Tyr Ala Ile Thr Val Glu Thr Arg Leu Ile Asp
65 70 75 80
Glu Arg Ala Ala His Val Asn Ala Gln Phe Arg Phe
85 90

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

Asp	Thr	Ile	Arg	Ile	Ala	Gln	Pro	Lys	Ser	Ala	Thr	Ala	Ile	Phe	Asp
1				5					10					15	
Thr	Thr	Thr	Leu	Asn	Pro	Thr	Ile	Ala	Gly	Ala	Gly	Asp	Val	Lys	Thr
			20					25					30		
Gly	Thr	Glu	Gly	Gln	Leu	Gly	Asp	Thr	Met	Gln	Ile	Val	Ser	Leu	Gln
		35					40					45			
Leu	Asn	Lys	Met	Lys	Ser	Arg	Lys	Ser	Cys	Gly	Ile	Ala	Val	Gly	Thr
	50					55					60				
Thr	Ile	Val	Asp	Ala	Asp	Lys	Tyr	Ala	Val	Thr	Val	Glu	Thr	Arg	Leu
65					70					75					80
Ile	Asp	Glu	Arg	Ala	Ala	His	Val	Asn	Ala	Gln	Phe	Arg	Phe		
				85					90						

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

Asp 1	Thr	Ile	Arg	Ile 5	Ala	Gln	Pro	Lys	Ser 10	Ala	Thr	Ala	Ile	Phe 15	Asp
Thr	Thr	Thr	Leu 20	Asn	Pro	Thr	Ile	Ala 25	Gly	Ala	Gly	Asp	Val 30	Lys	Ala
Ser	Ala	Glu	Gly	Gln	Leu	Gly	Asp 40	Thr	Met	Gln	Ile	Val 45	Ser	Leu	Gln
Leu	Asn 50	Lys	Met	Lys	Ser	Arg 55	Lys	Ser	Cys	Gly	Ile 60	Ala	Val	Gly	Thr
Thr 65	Ile	Val	Asp	Ala	Asp 70	Lys	Tyr	Ala	Val	Thr 75	Val	Glu	Thr	Arg	Leu 80
Ile	Asp	Glu	Arg	Ala 85	Ala	His	Val	Asn 90	Ala	Gln	Phe	Arg	Phe		

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Asp 1	Thr	Ile	Arg	Ile	Ala	Gln	Pro	Arg	Leu	Val	Thr	Pro	Val	Val	Asp
				5					10					15	
Ile	Thr	Thr	Leu	Asn	Pro	Thr	Ile	Ala	Gly	Ala	Cys	Asp	Ser	Lys	Ala
			20					25					30		
Gly	Asn	Thr	Glu	Gly	Gln	Ile	Ser	Asp	Thr	Met	Gln	Ile	Val	Ser	Leu
		35					40					45			
Gln	Leu	Asn	Lys	Met	Lys	Ser	Arg	Lys	Ser	Cys	Gly	Ile	Ala	Val	Gly
		50				55					60				
Thr	Thr	Ile	Val	Asp	Ala	Asp	Lys	Tyr	Ala	Val	Thr	Val	Glu	Thr	Arg
65					70					75					80
Leu	Ile	Asp	Glu	Arg	Ala	Ala	His	Val	Asn	Ala	Gln	Phe	Arg	Phe	
				85					90					95	

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

Asp 1	Thr	Ile	Arg	Ile 5	Ala	Gln	Pro	Lys	Leu 10	Ala	Lys	Pro	Val	Leu 15	Asp
Thr	Thr	Thr	Leu	Asn	Pro	Thr	Ile	Ala	Gly	Lys	Gly	Thr	Val	Val	Ser
			20					25					30		
Ser	Ala	Glu	Asn	Glu	Leu	Ala	Asp	Thr	Met	Gln	Ile	Val	Ser	Leu	Gln
			35				40					45			
Leu	Asn	Lys	Met	Lys	Ser	Arg	Lys	Ser	Cys	Gly	Ile	Ala	Val	Gly	Thr
			50			55					60				
Thr	Ile	Val	Asp	Ala	Asp	Lys	Tyr	Ala	Val	Thr	Val	Glu	Thr	Arg	Leu
65					70					75					80
Ile	Asp	Glu	Arg	Ala	Ala	His	Val	Asn	Ala	Gln	Phe	Arg	Phe		
				85					90						

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

Asp 1	Thr	Ile	Arg	Ile	Ala	Gln	Pro	Lys	Leu	Ala	Glu	Ala	Val	Leu	Asp
Val	Thr	Thr	Leu	Asn	Pro	Thr	Ile	Ala	Gly	Lys	Gly	Ser	Val	Val	Ala
Ser	Gly	Ser	Glu	Asn	Glu	Leu	Ala	Asp	Thr	Met	Gln	Ile	Val	Ser	Leu
Gln	Leu	Asn	Lys	Met	Lys	Ser	Arg	Lys	Ser	Cys	Gly	Ile	Ala	Val	Gly
Thr	Thr	Ile	Val	Asp	Ala	Asp	Lys	Tyr	Ala	Val	Thr	Val	Glu	Thr	Arg
Leu	Ile	Asp	Glu	Arg	Ala	Ala	His	Val	Asn	Ala	Gln	Phe	Arg	Phe	

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

Asp 1	Asn	Ile	Arg	Ile 5	Ala	Gln	Pro	Lys	Leu 10	Pro	Thr	Ala	Val	Leu 15	Asn
Leu	Thr	Ala	Trp	Asn	Pro	Ser	Leu	Leu 25	Gly	Asn	Ala	Thr	Ala 30	Leu	Ser
Thr	Thr	Asp 35	Ser	Phe	Ser	Asp	Phe 40	Met	Gln	Ile	Val	Ser 45	Cys	Gln	Ile
Asn	Lys 50	Phe	Lys	Ser	Arg	Lys 55	Ala	Cys	Val	Thr	Ala 60	Val	Ala	Thr	Leu
Ile 65	Val	Asp	Ala	Asp	Lys 70	Trp	Ser	Leu	Thr	Ala 75	Glu	Ala	Arg	Leu 80	Asn
Asp	Glu	Arg	Ala	Ala 85	His	Ser	Gly	Ala 90	Gln	Phe	Arg	Phe			

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

Cys Thr Thr Ala Thr Gly Asn Ala Ala Ala Pro Ser Thr Cys Thr Ala
1 5 10 15
Arg Glu Asn

Cys Ala Ser Gly Thr Ala Ser Asn Thr Thr Val Ala Ala Asp Arg Ser
1 5 10 15
Asn

Cys Phe Gly Val Lys Gly Thr Thr Val Asn Ala Asn Glu Leu Pro
1 5 10 15

Cys Phe Gly Arg Asp Glu Thr Ala Val Ala Ala Asp Asp Ile Pro
1 5 10 15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

Cys Gln Ile Asn Lys Phe Lys Ser Arg Lys Ala Cys Gly
1 5 10

Cys Gln Ile Asn Lys Met Lys Ser Arg Phe Ala Cys Gly
1 5 10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

Cys Gln Leu Asn Lys Met Lys Ser Arg Lys Ala Cys Gly
1 5 10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

Cys Gln Ile Asn Lys Phe Lys Ser Arg Phe Ala Cys Gly
1 5 10

(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

ATGAAAAAAC TCTTAAAGTC GCGGTTATTA TCCGCCGC

38

(2) INFORMATION FOR SEQ ID NO:106:

(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

ATGAAAAAAC TCTTGAAATC GGTATTAGTG TTTGCCGCTT TGAG

44

(2) INFORMATION FOR SEQ ID NO:107:

(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

ATGAAAAAAC TCTTAAAATC GGCATTATTA TTTGCCGCTG CGGG

44

(2) INFORMATION FOR SEQ ID NO:108:

(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

ATGAAAAAAC TCTTGAAATC GGCATTATTG TTTGCCGCTA CGGG

44

- (A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

ATGAAAAAAC TCTTGAAATC GGTATTAGCA TTTGCCGTTT TGGGTTCTGC

50

(2) INFORMATION FOR SEQ ID NO:110:

- (A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

TTAGAATCTG AACTGACCAG ATACGTGAGC AGCTCTCTCG

40

(2) INFORMATION FOR SEQ ID NO:111:

- (A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

TTAGAAGCGG AATTGTGCAT TTACGTGAGC AGCTC

35

(2) INFORMATION FOR SEQ ID NO:112:

- (A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

TTAGAATCTG AATTGAGCAT TAATGTGAGC AGCTCTTTCG TCG

43

- (A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

TTAGAATCTG AATTGACCAT TCATGTGAGC AGCTCTTTCA TTGATTAAGC G

51

(2) INFORMATION FOR SEQ ID NO:114:

- (A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

TTAGAAACGG AACTGAGCAT TTACGTGAGC TGCTCTTTCA TC

42